

# AIDS now a tractable disease?

Hopes that drug therapy will improve the lot of those infected with HIV have brightened, but it is too soon to tell what the consequences will be for the general spread of the disease.

AIDS has come to stay. So much is vividly illustrated by the inexorable increase of the numbers of those infected with HIV (human immunodeficiency virus), especially in the United States. When more than one per cent of the sexually adult population carries the infecting virus, and when there is no prospect of a downturn, it seems plain that the disease may well become as important a source of mortality among those in middle life as, for example, tuberculosis was in the 1930s. But there is also some good news. The spread of AIDS infection by heterosexual sexual intercourse appears not to have been as rapid as some feared just two or three years ago; at least in the short run, that is something to be grateful for. And now, there is the prospect that drug therapy may at least abate the development of overt AIDS in infected people. That many individuals may be helped to live longer, perhaps even to avoid the development of overt AIDS, is naturally welcome, but it is even more important that drug therapy could serve as a firebreak in the spread of infection, eventually limiting the social damage AIDS will cause.

But optimism should be constrained. Compared with the magnitude of the social problems already occasioned in places such as the United States by the spread of AIDS, the new developments (see *Nature* 340, 581; 24 August 1989) are but straws at which to clutch. The drug called AZT (otherwise the Wellcome drug known as 'Retrovir'), hitherto used in the palliation of overt AIDS, has been approved by the US Public Health Service as a prophylactic for use among those infected with the virus HIV. Experience so far seems to show that the onset of overt AIDS can be delayed, in about half those treated, for at least a couple of years (the duration of the trial). Then approval has also (and commendably quickly) been given for the preliminary testing in human beings of Genentech's clever synthetic protein, called immunoadhesin, made from molecules which combine the receptor molecule CD4 and part of the immunoglobulin molecule IgG1: but the first step is to tell whether the drug can be safely used in people: clinical trials proper will begin only in 1990.

Only the first of these developments can influence the immediate course of the spread of AIDS, and it is too soon to tell what the effects will be. For one thing, the use of AZT will be limited by the scarcity of the drug, based as it is on a natural product derived from herring sperm. Another way of putting that is to say that not many people infected by HIV and excluded from health insurance

schemes (often because of their infection) will be able to afford the annual cost of prophylaxis (in excess of \$6,000) out of their own pockets. Moreover, the wider benefits of prophylaxis remain to be determined. When so little is known of the infectiveness of people carrying HIV at the various stages between first infection and the development of overt AIDS, it is too soon to know the extent to which general use of AZT among carriers of HIV will reduce the spread of infection. (The bizarre possibility that, by prolonging the period of normalcy of the infected, general use of AZT might increase the potential for spreading infection cannot be dismissed, but seems unlikely.) On general grounds, if eventually shown to be effective, Genentech's immunoadhesin (which would remove circulating virus from the bloodstream) promises to have a more direct influence on the infection rate, but only time will tell.

The practical question remains of how these potential benefits may be secured not merely for those infected, but for society at large (which has a vital interest in any impediment to the spread of infection). The difficulties should not be underestimated. The physical scarcity of AZT is something to be reckoned with, and at the beginning will almost certainly limit the use of the drug to those carriers of HIV whose T-cells are already depleted (which is what the US Public Health Service recommends), but that will not extinguish demand from infected people (and their physicians) in whom there are no pathological signs. The cost of this development, especially in the United States, will be considerable.

Even if most of those infected with HIV are covered by health insurance schemes, so that the whole cost does not come from their pockets, their treatment will not be free, but will be reflected in the insurance premiums that everybody pays. At present prices, the aggregate cost might exceed \$1,000 million a year, by which yardstick Senator Edward Kennedy's proposal that there should be an extra \$30 million in the US budget to cover the cost of treating the uninsured seems over-modest. Yet this is an area of public expenditure in which, the US budget deficit notwithstanding, even the possibility that the wider use of AZT will be a firebreak in the spread of infection should be seized with energy — and at whatever cost is necessary. The emergence of drug prophylaxis, however insubstantial it may be at present, is one of the few cheerful developments in the past five years. □



# Conflicts of interest

The US Congress, now interested in conflicts of interest, should aim at a few prescriptive principles.

THE dispute that has come to light at the University of Pennsylvania (see page 668), and on whose rights and wrongs it is too soon to form an opinion, will at least provide further justification for the decision earlier this year of Congressman Ted Weiss, chairman of a congressional subcommittee to embark on a formal inquiry into conflicts of interest arising when academic research is supported both by industrial companies and by public funds. The inquiry is not merely proper but important. Yet nobody should jump to the conclusion that Weiss is about to uncover an area of academic life in which scandal is even more plentiful than the cases of outright fraud involving the misrepresentation of data that have been uncovered during this decade.

That commerce and academic research do not mix easily was dramatized by the first wave of excitement about the potential of biotechnology in the late 1970s, and has since been sharpened by the way in which governments have been urging on the academics who look to them for support that industrial interests should be more fully catered for in universities and research institutes. There are several pitfalls into which academics and their institutions may fall, ranging from the possibility that particular industrial companies may benefit unfairly from academic research projects (perhaps securing their advantage by appropriately genteel kickbacks) to the danger that the quality of research may be compromised (or its publication unduly delayed) by commercial interests.

To recite the sources of difficulty is not to argue that commercial interests have no place in academic laboratories. And since the social function of academic institutions is at least partly economic, it may be correctly argued that academic research institutions have a social duty to assist industrial companies in innovation and competitiveness. Striking a balance is naturally difficult, while it is plain that some governments (the British in the past decade, for example) go too far in their utilitarian demands.

These are issues for institutions to argue out with their sponsors. What interests the Weiss committee, properly, is whether commercial interactions affect the behaviour of individuals in research, with consequences that are either unseemly or downright inequitable. It will uncover in the months ahead, a lot of gossip, but its true goal should be to illuminate the general principles on which relationships between universities and industrial or commercial sponsors are regulated.

First, explicitness should be the general norm. If academics, singly or collectively (as a university department, for example), come to an arrangement with outside interests about their programmes of research, its basis should be fully disclosed. In one form or another, most

academic institutions are in a position to claim that this is already done: there are usually formal limits on the amount of time academics can devote to consultancies and rules requiring that at least some official of the university should be kept informed. But rules of this kind are often inadequately policed, and are insufficient.

Disclosure should serve several purposes, of which one is that a researcher's immediate colleagues understand what he is about. It would, for example, be corrosive of trust in research that one member of a research group should not have told his colleagues in the same academic enterprise of an outside connection with some commercial company. Yet there are many universities in which the rules do not require even that degree of disclosure. And there is a general reticence about the sums of money that change hands. People may often satisfy the local regulations by disclosing that they have an outside arrangement, but may not say how much it is worth to them. The consequence is that the abatement of excess that colleagues might informally bring about does not apply. The general rule should be that disclosure should be full, and made public within institutions.

A second principle is that of how the financial rewards of outside commercial interests should be shared. As things are, most academic institutions leave the negotiation of outside arrangements to those concerned and let them keep the proceeds. If a university department takes on a research project for an outside company, it will usually recover the cost involved and an accompanying contribution to its overheads, but some of those carrying out the research may be rewarded separately. The matter of patent rights to important innovations is potentially even more contentious: while some universities, especially in the United States, have worked out arrangements by which they and successful inventors share the rewards of innovations, others have neglected the need to formalize these matters, to the general discontent. Nobody suggests that academics should not be paid, but there are merits in the simple rule that external earnings should be divided three ways, between the individual concerned, his department or research group (to support further research) and the whole institution. If the Weiss committee can win general acceptance of some such rule, it will do a public service.

A third principle concerns academic researchers who may become officers of independent commercial companies. In the past few years, people with a bright idea have sought to exploit it commercially by setting up a company, often appointing themselves as chairman, perhaps even chief executive. That is not merely unwise but also a serious threat to people's academic integrity. When companies run into serious trouble, their managers have a fiduciary duty to shareholders to drop everything else, doing what they can to save the commercial enterprise which is not compatible with academic life. The rule should be that academic researchers may be shareholders or non-executive directors, but never have management responsibility. Weiss should push for such an understanding. □



# Voyager's final Solar System rendezvous

- High clouds and hurricanes on Neptune
- Triton marked by glaciers and ice volcanoes

## Pasadena, California

As Voyager 2 rounded Neptune 5,000 km above the clouds and sailed within 25,000 km of the large moon Triton, it ended its spectacular 12-year mission with a flourish.

Neptune, the last of the giant gaseous planets in the Solar System, turned out to be a more visually appealing object than the generally featureless blue globe of Uranus. On Neptune, Voyager saw high cirrus clouds and a number of large visible features, some bright and some dark, that maintained their identity over the duration of close approach. And Voyager's close pass by Triton revealed a small world marked by recent and probably continuing activity: despite the intense cold, which makes water ice on Triton as unyielding as rock on Earth, Neptune's biggest moon is covered by flows, frozen lakes and 'icy volcanoes'.

Voyager 2, like Voyager 1, will now coast on towards interstellar space, and should remain in radio contact for another thirty years. It will send back measurements of the intensity of the solar wind, and may eventually cross the heliopause, where true interstellar space takes over from the region dominated by the influence of the Sun, but there is nothing more to take pictures of. At Voyager mission control, the Jet Propulsion Laboratory (JPL) in Pasadena, California, Vice-President Dan Quayle came to give the spacecraft a send-off, and as the stream of data from the encounter dwindles, the specialist teams will return to their home institutions to puzzle over their new discoveries. JPL is now turning its attention

to the future (see page 669).

The atmospheric activity found on Neptune came as a surprise. Uranus, about the same size as Neptune and similarly shrouded in a dense methane atmosphere, presented a bland face to Voyager three and half years ago, and there was reason to think that Neptune, half as far again from the Sun and correspondingly colder, would be equally inactive.

But even a few months before the encounter, images from Voyager began to reveal large and conspicuous markings on Neptune. The most prominent became known as the Great Dark Spot, in recognition of its superficial similarity to Jupiter's Great Red Spot, and as Voyager neared its destination, a handful of other features appeared. A smaller dark spot moves around the planet close to the south pole, and as it grew in size on successive images, a bright spot appeared in its centre. Between the Great Dark Spot and the smaller one is the 'scooter', a small bright feature that moved around Neptune once every 16 hours, rapidly overtaking the dark spots, whose rotation period is closer to 17 hours.

Scattered across Neptune in a number of latitude bands are bright streaks that were instantly and fittingly described as cirrus clouds. Although a handful of cirrus features appear around the Great Dark Spot in fairly fixed positions, most of the high bright clouds changed from one rotation to another. This gave atmospheric dynamicists a problem: to estimate the circulation speed of the atmosphere, they need to see features that can be ►

## Navigating to Neptune

ON 21 August, 4 days and 6 million km before closest approach, Voyager 2 made its final course adjustment, a sideways nudge in velocity of half a metre per second in an overall speed of 20 km per second. This delicate change, shifting Voyager's arrival point at Triton by 200 km, put it directly behind the moon with respect both to the Earth and the Sun so that two occultations could be observed.

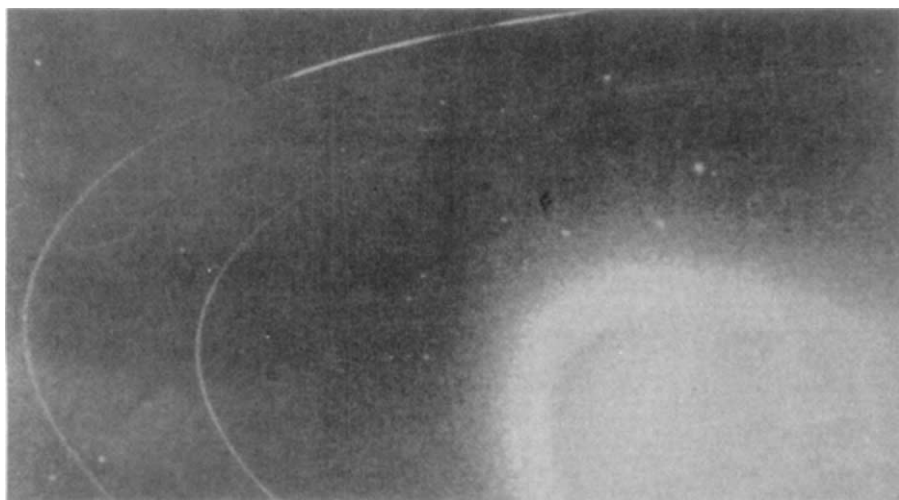
The apparently easy success of this fine-tuning of the trajectory conceals 12 years' worth of practised ingenuity on the part of the JPL scientists and technicians. Voyager's main thrusters, normally used for steering, cannot be fired during a planetary encounter because they heat up the radio receiver and, because of a long-standing malfunction, would cause a loss of contact with Earth for two or three days until the electronics cool down again.

During a planetary encounter, Voyager is steered instead with its attitude adjustment thrusters, which normally fire automatically every 20 minutes or so to keep the radio antenna pointed to Earth. Firing one of these sets Voyager spinning about the axis of the radio dish; when half a turn has been completed, another thruster can be fired to set the spacecraft rotating back the same way. Finally, the first thruster is fired again to stop rotation. The rocket firings are all in the same direction, perpendicular to the direction of motion; the net effect is to edge Voyager crablike across the sky, without loss of radio contact.

Only after this manoeuvre, when Voyager's passage through the neptunian system was calculated to an accuracy of 30 km, was the final set of instructions for the encounter prepared and transmitted. Some retargeting of the cameras was done to get close-up shots of two of the newly discovered moons, N1 and N2, and of segments of the rings.

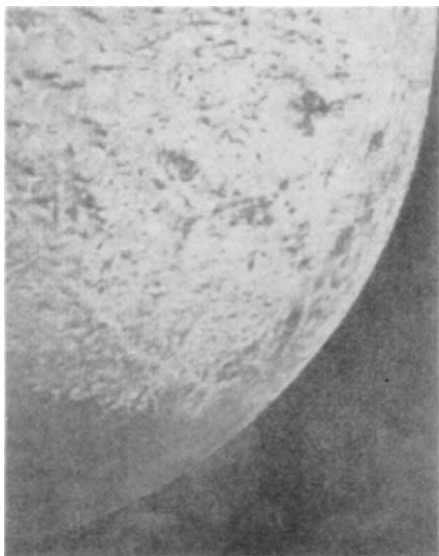
On Wednesday afternoon, 23 August, Voyager's instructions for the encounter were transmitted. Because of the eight-hour interval between sending signals and receiving confirmation of their arrival, the command sequence was sent six times to ensure error-free receipt. Voyager's memory, state-of-the-art 12 years ago, can hold only 2,000 words of instructions: during the encounter, when the detectors must be turned constantly from one target to another, the meagre space is barely enough to hold two days' worth of instructions.

The finishing touch was delivered on Thursday morning, about 12 hours before closest approach. From final course determinations, an exact set of timings for the encounter was calculated and transmitted, telling Voyager when to execute the already installed series of commands. From this time until late Saturday afternoon, when it would be 2,500,000 km beyond Neptune, Voyager was on its own. □



Surprises included five rings (two seen here) and a magnetic pole 50° off the rotation axis.





Triton: there's no such thing as a 'dead world'. unambiguously identified over several rotation periods. Around the Great Red Spot of Jupiter, for example, a multitude of recognizable smaller clouds pass by, permitting the rotation sense of the spot to be determined. But around Neptune's Great Dark Spot, the pattern of cirrus clouds is different on every image. Several days after the encounter, it was still impossible to decide if the Great Dark Spot is a cyclone or an anticyclone; as on Earth, the sense of rotation is different according to whether the spot is a low- or high-pressure region.

On some remarkable pictures, cirrus clouds are seen to cast shadows on the basal cloud layer; the height of the cirrus layer was estimated at about 50 km. The dark spots appear to be about halfway between the high and low cloud layers.

The high clouds are methane condensations; the lower clouds are thought to consist of hydrogen sulphide. Sunlight acting on methane in the upper atmosphere probably creates heavier hydrocarbons, which freeze and sink; lower in the atmosphere, where the temperature rises again, they evaporate and decompose back to methane, which rises in plumes to form the visible clouds. On Neptune, further from the Sun than Uranus, most of the energy available to drive atmospheric dynamics is internal heat, welling up from below, rather than solar energy impinging on the upper atmosphere.

Passing beyond Neptune, Voyager 2 saved some of the most startling images of its career for last. Triton, a cold and presumably icy place 2,700 km in diameter, was far from dead. At a quick glance, cratering on Triton seems not to be heavy, implying that the surface has been processed somehow in fairly recent times — the past 100 million years, for example. There had been suggestions that Triton would look 'new'; it has a uniquely odd orbit, circular but inclined to Neptune's axis, and retrograde. This makes it almost

certainly a captured body, and suggests that in earlier times its orbit was probably not circular. As tidal forces circularized Triton's orbit, the strains on the moon's body would have heated it up, perhaps enough to melt water ice.

This indeed seems to have been the case. Parts of Triton's surface seem to have 'frozen lakes', resembling lunar maria. In places, the frozen lakes are stepped, suggesting a succession of meltings and re-freezings. But there is much more. Triton's inclined orbit, coupled with Neptune's, means that over a seasonal cycle of about a thousand years the Sun can be overhead anywhere from 50 degrees south to 50 degrees north. At the moment of Voyager's fly-by, the Sun was far to the south as seen from Triton, and near its south pole the surface markings were reminiscent of a martian summer, when the Sun is warm enough to evaporate the frozen carbon dioxide polar caps. On Triton, the ices are methane and nitrogen, but the process seems to be the same. Methane ice on Triton lends it a pinkish colour, darkly mottled by radiation damage from high-energy ionized particles which stream onto the moon's surface and create darker hydrocarbons from the methane.

Elsewhere there is evidence of smaller flows that have filled in valleys and fissures. These are probably glaciers of methane and nitrogen which, like ice glaciers on Earth, can flow slowly over millions of years. Most bizarre of all are a few dark elongated streaks, tens of kilometres across. Initial suggestions that these might be wind-trails, formed of evaporating surface ices dispersed by the atmosphere, did not last long. Triton's atmosphere, inferred from the observation of a stellar occultation, has a pressure of no more than 10 millionths of Earth's, and is mostly nitrogen. There is too little of it to create a trail of sublimed ice.

The explanation settled on a few days after encounter was that the trails were from 'ice volcanoes'. Because nitrogen is just barely frozen at the surface temperature, an overburden of 20 or 30 metres is enough to cause nitrogen ice to liquefy. If fissures develop in the overlying ice, the liquid below will burst out, turn to gas which can shoot several kilometres above the surface, then condense and fall back. The thin surface layer so formed appears dark only in comparison with its surroundings, and may also entrap some of the sublimed dark ice particles in the vicinity.

A similar process may also account for yellow streaks seen on Io, one of Jupiter's moons, where underlying sulphur rather than nitrogen may rush up through surface cracks. Triton would then seem like a jigsaw of pieces from elsewhere in the Solar System, harbouring analogues of the martian polar caps, lunar maria and Io's vulcanism.

David Lindley

## Rings, arcs and moons

BEFORE Voyager got close to Neptune, many astronomers were anticipating confirmation of the existence of many 'ring arcs', or partial rings. But by early this week, only four or five rings had been found, and all were complete, encircling the entire planet. Where did the arcs go?

Observations from Earth of occultations of stars passing behind Neptune suggested partial rings: stars were seen to flicker in brightness as they approached Neptune's disk, but no diminution in brightness was seen as the star emerged at the other side.

Altogether there was evidence for six ring arcs, at various distances from the planet. The early signs from Voyager looked good. JPL scientists announced three weeks before the encounter (see *Nature* 340, 492; 1989) that they had detected faint reflected light from two partial rings.

But on Tuesday 22 August, new images revealed that the inner arc was in fact a complete ring, and that the other arc was longer than first thought. Much better pictures came down on Saturday, after closest approach, when Voyager could look back to see the ring system shining brightly in forward-scattered rather than reflected sunlight.

The full ring system revealed in these backlit images is complex. The two bright rings, 53,000 and 62,000 km in radius, are indeed both full rings, but the outer one has conspicuous clumps. Inside the inner bright ring is a faint and rather wide band, 42,000 km from Neptune and 2,000 km wide. Between the two bright rings is a fainter ring, but rather than a distinct ring this may be the edge of a tenuous, barely visible disk of material extending inwards to the inner bright ring, and possibly beyond.

The final assessment was that ground-based observers had indeed detected clumps in the outer ring — three of the observations gave about the right radius — and made the simple assumption that the ring was incomplete.

But one occultation measurement was both too far out and too strong to be consistent with any observed ring. Despite the astronomical odds, it seems that this occultation was caused by the then unknown moon 1989N2, a 200-km-wide body happening, at a distance of 500 million km, to pass in front of a background star.

The final haul of moons was six, varying from 42,000 km out and 50 km in diameter to 120,000 km out and 500 km across. As with the other giant planets, the rings and moons form a complex system, whose properties will take years to puzzle out. Neptune's clumpy outer ring demands explanation, but none is immediately to hand: none of the moons is in the right place or has the right mass to be clearly responsible, by its gravitational influence, for the non-uniformities observed. □

## Boost for Antarctic research

### Sydney

IN line with the prime minister's concern for the environment, the Australian government has greatly increased support for research in the Antarctic and on the greenhouse effect in its annual budget. One hundred and thirty-five projects, 10 per cent more than last year, will be allocated \$59.8 million for the 1989-90 year. This is an increase of almost \$16 million. About a quarter of the projects focus on research relating to the greenhouse effect, depletion of the ozone layer, research into the history of climate change and future climatic trends.

Much of the allocation will go toward a new Antarctic air-transport system to be tested over the summer months. Trials for an intercontinental Australia-Antarctica air link will involve two Royal Australian Air Force Hercules flights from Hobart in Tasmania to an ice runway at Casey station, one of three Australian permanent year-round research stations in the Antarctic. If successful, the trials may open the way for the introduction of a permanent Antarctic air-transport programme by 1990-91.

At present, travel to Antarctica from Tasmania can take up to six weeks by sea. According to Rex Moncur, acting director of the Antarctic Division of the federal Department of the Environment, an air link will not harm the environment. "Unlike the French, we are not using a rock platform, but an ice runway. The 2 per cent of the land mass that is rock is the major habitat of wildlife, whereas there is little wildlife on the ice. We will, however, conduct a two-stage environmental impact

study just before the trial and a more detailed one before any full-scale air operation. An air link would mean that we could design research projects without having to consider whether we can get personnel or equipment in rapidly, and it will also encourage more senior people to do research in the Antarctic."

Funding has also been provided for the chartering of the marine research and supply ship, *Aurora Australis*. Since the sinking of the *Nella Dan* in 1987, Australia has been without a fully equipped marine research vessel. Two supply ships have been providing transport for passengers and cargo to and from the continent, but they have only limited research capabilities.

Unlike any other ship working the Antarctic, the *Australis* is designed to have no fuel tanks in its outer hull, making an oil-spill unlikely even in the event of an accident.

One of the first projects earmarked for the *Australis* will be to determine the amount of damage done by commercial trawling, particularly by Japanese boats. Other projects, both continuing and new, include investigations of the effect of increased ultraviolet radiation and carbon-dioxide levels on Antarctic and sub-Antarctic plants; research designed to limit the environmental impact of Australia's Antarctic research stations; analysis of ice core to determine the composition of the Earth's atmosphere in past ages; and, finally, studies of seasonal variations in atmospheric aerosol concentration and gas emissions from Antarctic waters.

Tania Ewing

### HUMAN GENOME

## Support for Japan's sequencers from MESC

### Tokyo

THE Science Council of Japan's Ministry of Education, Science and Culture (MESC) has decided to give a small boost to attempts to start a human genome project in Japan. But launch of a full-scale project is still a long way off.

In line with recommendations received from the Science Council earlier this month, the ministry is to provide ¥600 million (about \$4 million) for a two-year preparatory study to be led by Kenichi Matsubara of Osaka University. The money, the first financial commitment to the human genome project by the ministry, will be released in September from 'emergency' funds, a source which shows that the ministry considers the project to be a matter of some urgency, says Matsubara.

The sum is comparable to that already being spent by the Science and Technology Agency to develop an automatic DNA-

sequencing machine.

A decision on how the money will be used is not expected until next month. But Matsubara hopes that some of it will go to improve repositories and computer facilities, such as the DNA data bank at the National Institute of Genetics in Mishima.

The preparatory study, which will be carried out by about 20 researchers, including computer experts and biologists, will look at three key issues: training and recruiting of research staff, dissemination of results and ethical issues of genome sequencing.

Matsubara hopes to recruit many more researchers to the project over the two-year period. But he says that some researchers are concerned that the project may drain resources from other fields and he is trying to raise money from private industry and other sources.

David Swinbanks

## US opposition to milk hormone

### Boston

IN response to pressure from farm organizations and environmental groups, five of the largest supermarket chains in the United States agreed last week that their house brands of dairy products would not contain milk from cows injected with the genetically engineered hormone bovine somatotropin (BST).

The boycott by 2,500 supermarkets in the United States follows heated debate in Europe (see *Nature* 340, 415; 10 August 1989) and comes despite US Food and Drug Administration (FDA) approval for experimental use of the peptide hormone. When BST is injected into cows once or twice a month it can increase milk production by as much as 30 per cent. More than a hundred cattle herds have tested the hormone in the last four years.

FDA has yet to license BST fully for marketing and routine use but milk and meat from experimentally treated herds can be sold for human consumption. FDA representative Bonnie Aikman says that the data upon which that decision was reached will be published early next year in a scientific journal.

The announcement by the food companies came in response to a letter, challenging the companies to set out their positions, from the Foundation on Economic Trends, the group led by Jeremy Rifkin.

Paul Bernish, public affairs director at the Kroger Company, the largest US supermarket chain, affirms his company's belief in the role of the FDA to test new products to insure a safe food supply. But Bernish says "prudence" dictated his company's decision. "Until the government reaches a final decision", he says, "we simply would prefer not to have such products in our milk supply." Earlier this summer, the Vermont legislature called for a moratorium on the commercial use of bovine growth hormones pending a congressional investigation of its impact on farmers and consumers. Other major dairy states have introduced legislation to ban hormone-treated milk.

Rifkin's group and some 40 other farm and environmental organizations last week petitioned the FDA to stop all sale of BST-treated milk until final licensing is approved. They asked the FDA to reveal the location of test herds and to undertake a long-term study that will take into account the health of the nation's cattle, the possibility of long-term effects on humans and the economic impact on dairy farmers.

Aikman says that the FDA has yet to review the petition formally. But she stresses that the agency has no right to examine the economic effects of BST use. "We're here simply to rule on the issue of the product's safety." Seth Shulman



# Bitter dispute reaches NIH

## Washington

NEXT month, an investigative committee from the National Institutes of Health (NIH) will be set up to look at one of the most tortuous biomedical controversies of the 1980s. Ostensibly, the committee will have the task of scrutinizing the scientific merits of a clinical trial of an antibiotic used to treat infection of the middle ear in children. But the dispute over the research, conducted at the Otitis Media Research Centre (OMRC) at the Children's Hospital of Pittsburgh, now involves allegations of misconduct and financial conflict of interest, and raises questions of the ownership of federally funded research and the proper role of scientific journals and university inquiries in the resolution of scientific disputes.



Charles Bluestone — under fire.

As in other quarrels that NIH have investigated, the two protagonists in the dispute — Professor Charles Bluestone, director of OMRC, and Professor Erdem Cantekin, former director of research for pediatric otolaryngology at the hospital — do not agree on even the simplest facts. They have not spoken to one another since 1986. At that time, Bluestone, who had asked Cantekin to join the department 11 years earlier, removed him from his post as research director. Cantekin now sits alone in an office at the end of a quiet corridor far removed from his former colleagues. Having faced three university investigations and a congressional hearing, Cantekin now leads the life of a full-time defendant.

The quarrel began with a scientific argument over the results of the trial. Cantekin claims that the research shows that the antibiotic under test, amoxicillin, is not effective in treating infection of the middle ear when compared with a placebo. He says that the results are flawed because the researchers relied too heavily on the physical observation of the ear with an otoscope, a technique which he believes is prone to observer bias.

Bluestone says that the antibiotic is

effective and claims that Cantekin's analysis is flawed, in part because the endpoint of the trial was determined after the data were collected.

## Who owns the data?

The dispute became public when, having failed to settle their differences, both researchers sent manuscripts to the *New England Journal of Medicine (NEJM)*. Faced with two views of essentially the same data, the editor Arnold Relman, decided that the journal was "in no position to make any judgement between them". Instead he wrote to the University of Pittsburgh for instructions as to which was the 'authorized' version.

Professor Eugene Myers, now dean of the University of Pittsburgh School of Medicine, co-signed a letter to Relman to express his "total and complete support for, and endorsement of" the Bluestone manuscript, which was the "only authorized manuscript". Cantekin's manuscript was rejected and Relman explained in a letter to Cantekin's lawyer that "the important question is . . . not whose interpretation is correct . . . but rather who has the right to publish the data first". Subsequently, *The Lancet* also rejected the manuscript, suggesting that it be published as a letter. But the *Journal of the American Medical Association (JAMA)* expressed "substantial interest" in the manuscript.

Answering questions at a congressional subcommittee hearing on conflict of interest in June, the editor of *JAMA*, George Lundberg, said that to ask an institution to say who are the "appropriate" authors "would undermine the trust relationship" between editors and authors. In the unlikely event that a journal should receive two manuscripts analysing differently the same data, it should review them both, he said. A journal should publish "the most truthful and important articles possible without being particularly concerned as to what the institutional politics might be". Cantekin's manuscript was reviewed by *JAMA*, but by the time the review process was complete, the university had begun misconduct proceedings against Cantekin. *JAMA* refused to publish the paper until these were over.

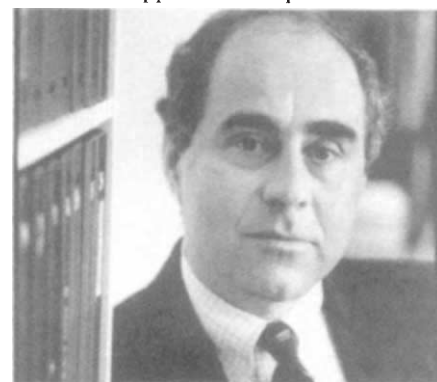
The misconduct hearings began when Cantekin accused Bluestone and Myers of obstructing his right to publish freely the results of publicly funded research, and of punishing him for his dissenting views. But these charges were dismissed and counter-charges of academic misconduct were made by Myers against Cantekin. Two university panels investigating the charges concluded that submitting the manuscript to the *NEJM* was unethical.

The investigations entered the final

stages this month when Cantekin appealed to Wesley Posvar, the president of the university. But regardless of what he decides, the university may be unable to discipline Cantekin further. To strip him of tenure and remove him from the faculty would require the consent of the university senate. But the senate's committee on tenure and academic freedom has said that the School of Medicine's research integrity policy "puts the power of judgement in the hands of those most anxious to preserve a *status quo*". The committee in turn has been accused of bias by Myers, who says it has "never listened to the other side of the story" and is "always on the side of the underdog".

## Conflict of interest?

Bluestone's behaviour has since become subject to further scrutiny after Cantekin claimed that he had failed to declare fully financial support from pharmaceutical



Erdem Cantekin — vocal critic.

companies, as required by NIH.

No money was received for the disputed trial, but OMRC has received large sums since its foundation. The research carried out there is important to the pharmaceutical companies because infection of the middle ear is one of the most common childhood ailments. Doctors routinely prescribe antibiotics for it, at a cost in the United States of about \$500 million a year.

The university says that in obtaining funds from pharmaceutical companies, Bluestone is "within the university guidelines" and that there is no concern about a conflict of interest. But a committee from NIH, which also looked into the allegations, recently sent to the director an interim report saying that it "found merit in the allegation that OMRC has not generally disclosed to NIH the extent of its industry-sponsored research". The report, which has not been made public, says that although a few studies supported by pharmaceutical companies were cited specifically, "no dollar amounts were given". But it also says that many institutions were found to interpret the instructions for grant applications improperly at that time.

The committee found that from 1983 to 1988, Bluestone received almost \$300,000



## NEPTUNE ENCOUNTER

# JPL looks beyond Voyager

## Pasadena

As the Voyager 2 spacecraft sped past Neptune last week and off into the furthest reaches of space (see page 665), it marked the end of the first grand reconnaissance of the Solar System. It also highlighted a new era for the Jet Propulsion Laboratory, which manages the Voyager mission and many other unmanned space expeditions for NASA (the National Aeronautics and Space Administration).

After a stormy period of budget cuts, JPL is basking in the warmth of President George Bush's support for space studies and is preparing itself for a new phase of exploration that will dictate its direction well into the next century.

Begun in the 1930s as an aeronautical laboratory for nearby California Institute of Technology (CalTech) and later serving as an Army research facility, JPL was turned over to NASA when the agency was created in 1958. Its assignment was to take the lead in the unmanned exploration of the Solar System. Besides the twin Voyagers, early missions included the Mariner series that explored the inner planets and the Viking journey to Mars. But while scientists are still reaping the benefits of these missions, the launch of the last Voyager in 1977 meant that JPL had to look to new venues for its bread and butter.

The time of the Voyager launch coincided with a period of waning federal support for the space programme that reached its nadir with budget and staff cuts in 1982. The crisis prompted a decision to accept funds from other government agencies — most notably the Department of Defense — and set off an uproar at both JPL and CalTech, which through a unique arrangement pays the salaries of the laboratory employees. But the move bolstered JPL's research base. And since 1983, the facility has enjoyed steady increases in support that have brought its

## ANIMAL RIGHTS

### Monkeys go home

#### Paris

A LYONS tribunal decided on Tuesday 22 August to order the return of 28 monkeys to the research laboratory from which they were stolen in May. The monkeys had been held at a primate centre in Strasbourg. Meanwhile, eight members of an anti vivisection organization, Arche de Noé, are awaiting trial for the break-in.

During the night of 19 August, the Front de libération des animaux, broke into kennels in northern France, releasing 28 beagles belonging to a small company which carries out toxicity trials on drugs intended for humans. Peter Coles

annual budget to a little over \$1,000 million and its staff to 5,200, of whom more than half are engineers and scientists.

The laboratory's strategy for the rest of the century and beyond focuses on three general areas — additional Solar System studies, investigation of the Earth itself and studies of other solar systems.

The first category includes some missions planned long ago but only now getting under way. Among them are the Galileo mission to Jupiter, which has an October launch date, and the Mars Observer, which is scheduled for 1992. Both are projects the laboratory had hoped to complete in the early 1980s. Future plans include the Cassini mission to Saturn, which will be the first JPL craft to employ 1980s technology. Also in the planning stages are efforts to build automated rovers that would land on planets and satellites and return surface samples to Earth. "We are now getting into a new phase of science — the 'in situ' robotic exploration of the Solar System", says the laboratory's chief scientist, Moustafa Chahine.

For most of JPL's history, Earth had been ignored. Ten years ago, the laboratory formed an Earth and Space Sciences Division. More recently, JPL scientists began working on the Earth Observing System (EOS), a gigantic undertaking involving at least two space-based platforms, the first of which is due to be launched in 1996. All told, JPL scientists designed and developed 11 instruments — nearly a third of the total payload — that will fly aboard the two EOS platforms.

Each step in JPL's evolution has meant new developments in microelectronics, information systems, image processing and computer systems to help analyse the data gathered. One new system involves the hypercube, a parallel computer pioneered at CalTech. JPL scientists are working on a system, Mark 3fp, that interconnects up to 128 processing nodes that can each tackle the same or different tasks. The system will be able to combine data from different sources — microwaves, radio waves, infrared and visible light — into a multi-dimensional image that make it possible to see directly patterns in data that are normally hidden in piles of computer print-outs.

Despite such new projects, some critics say JPL is showing its age. Director Lew Allen disputes that vigorously, saying a crop of new scientists has moved in alongside the laboratory veterans. He is more worried that the long gaps between missions have left his crew rusty. Since one mistake can cost years of planning, he notes, "you clearly worry a great deal about whether your skills are up to snuff".

Robert Buder

in honoraria, about \$260,000 of it from pharmaceutical companies. And since 1980, it says, OMRC has received almost \$3.5 million from pharmaceutical companies. The committee says this "gives the appearance of a conflict of interest".

But the committee found no favourable bias toward drug companies in the research results, largely on the grounds that the "the number of positive and negative studies were approximately equal".

Bluestone says he did not disclose the amounts he received because he "misinterpreted" the NIH instructions, but he claims that full descriptions of the industry-funded studies were described in progress reports. Speaking at the June congressional hearing, James Wyngaarden, former director of NIH, said that before 1985, the instructions were not clear. When they were made more explicit, he said, it was "quite possible that he [Bluestone] just didn't notice the new requirements and neither did we".

## Scientific dispute

The new NIH committee now has the difficult task of unravelling the scientific issues in the dispute. Wyngaarden said that "some of the rather unusual steps Cantekin has taken might be viewed somewhat differently if his scientific views are sustained". But an independent consultant who reviewed the manuscripts for the university hearing board said "it may be that replication of the trial is the only way to resolve the current dispute". Congressman Ted Weiss, chairman of the subcommittee that held the June hearing (see *Nature* 339, 568; 1989), argues that the results of a subsequent study by OMRC also appear to show that amoxicillin is no more effective than a placebo. Bluestone denies this and says that the results were taken out of context. He also denies allegations made by Cantekin that he has intentionally delayed publication of the results. He claims that the manuscript is being submitted to *NEJM* this week. Cantekin asked to see a final report on the study prepared for NIH but his request was denied because the contents are patentable. The study was funded in part by the companies whose antibiotics were being studied.

Weiss is not satisfied with written answers he has received from Bluestone and plans to hold a hearing in the next few months at which Bluestone will testify for the first time. This case, he said "provides us with many examples of what should not be happening with NIH-funded studies". With its particular interest in how institutions handle misconduct cases, and in ensuring that whistleblowers are protected, the House of Representatives subcommittee on oversight and investigations, chaired by John Dingell, is also following the controversy closely.

Christine McGourty

# Japan ends its isolation

## Tokyo

TOKYO University this month opened the first high-capacity computer link between Japan's national research institutes and scientific computer networks in the United States and other parts of the world. The link is expected to stimulate development of Japan's primitive computer networks and may also help to break down some of the bureaucratic barriers that exist between Japan's research institutes.

The new computer link, the Todai (Tokyo University) International Science Network, was established after Professor T. Nielson of the Department of Computer Information Science at the University of Hawaii approached Tokyo University's faculty of science late last year in search of a landing point in Japan for his Pan-Pacific Computer Network.

Unable to get government funding for the network, Akiyoshi Wada, dean of the faculty of science, with the help of Ken Sakamura of the university's department of information science, arranged for the giant computer manufacturer Fujitsu to support the project. Fujitsu has donated workstations for the network worth about ¥30 million (\$210,000) and for the next 18 months the computer company will cover the 1-million-yen-a-month cost of leasing a high-capacity (64 kilobits per second) line between Tokyo University and Hawaii University.

The network will provide access to most

of the scientific networks in the West such as HEPNET, used by high-energy physicists, SPAN, a network for astrophysicists, and LIFENET, used by life scientists. And Japanese scientists will be able to log onto computers in research centres in the United States and Europe as if the computer were "in their own room", according to Tsuneyoshi Kamae, chairman of the committee which established the network. Similarly, Western scientists will be able to log onto computers in Japan.

About ten national research institutes, including the High Energy Physics Laboratory (KEK), the Institute of Physical and Chemical Research (RIKEN), the Institute of Space and Astronautical Science (ISAS) and the Institute of Genetics, are expected to use the network, and these institutes and Tokyo University will pay for the network's running costs when Fujitsu's support runs out.

Surprisingly, the major running cost may not be the link with Hawaii but rather some of the domestic links in the network. The opening up of Japan's international telecommunications market to two new companies, including one backed by Cable and Wireless of the United Kingdom, has forced the former monopoly Kokusai Denshin Denwa (KDD) dramatically to reduce its rates. Even during the few months of planning the network, KDD reduced the price of the Tokyo-Hawaii link by more than 20 per cent.

But domestic telecommunication rates are still maintained at inflated levels by Nippon Telegraph and Telephone (NTT), despite the recent establishment of several small competing domestic telecommunication companies. For example, leasing an NTT line between Tokyo University and the Institute of Genetics in Mishima, a distance of about 120 km, costs more than half as much as the 10,000-km link to Hawaii.

Organizers of the new network hope to circumvent this problem by using the computer network of the National Center for Science Information System (NCSIS) which links Japan's universities and inter-university research institutes. NCSIS charges much lower rates than NTT but the capacity of its network (9,600 bits per second) is too limited. Tokyo University and KEK researchers, however, are requesting that it be upgraded to 48 kilobits per second.

Kamae says that computer networks in Japan lag far behind those of the United States and Europe. Computer companies and government ministries, he says, still maintain a "wartime attitude" about computers. They build huge mainframes "like the battleship *Yamato*" but not the small workstations that best suit computer

networks. The workstations supplied by Fujitsu are made under licence from the US company Sun.

The Japanese government has built many huge "monuments" to science in the form of institutes and universities, Wada says, but "just as the navy built the battleship *Yamato* and forgot about logistics and communications", the government has failed to link Japan's research organizations into a coherent whole.

At the root of the problem is inter-ministry and inter-agency rivalry which prevents institutes and researchers belonging to different government organizations from interacting. But the new computer network seems likely to break down some of these barriers.

RIKEN is anomalous among the organizations joining the new network because it belongs to the Science and Technology Agency; the other institutes and the University of Tokyo are all affiliated to the Ministry of Education, Science and Culture (MESC). RIKEN will not be able to use the MESC-funded NCSIS network. Instead, a more expensive NTT line will be used. But by building up the number of users of the Todai network, Wada and Kamae hope to bring down the costs of subscription to a level that any institute or university in Japan can easily afford without recourse to special government support.

**David Swinbanks**

## NUCLEAR POWER

### Alarm at plants

#### Paris

SERIOUS anomalies have been found this month in safety devices built into two French nuclear electricity generating plants. In both cases, temporary modifications to safety circuits were left in place after routine maintenance, rendering them essentially ineffective in the case of an accident. The first anomaly was detected earlier this month at the Dampierre plant.

Two plugs, fitted for tests on piping in the containment architecture, were not subsequently removed. Circuits designed to reduce the risk of an explosion in the event of a major accident were thus apparently compromised.

The most recent fault was discovered last week at the Gravelines reactor and was rated as a Class 3 incident (on a scale of six). Three safety valves, designed to prevent pressure build-up within the reactor in the case of an incident, were fitted with screws of the wrong type. It emerges that the screws had been in place since June 1988. The central nuclear installation safety service (SCSIN) has said that it is concerned by this apparent negligence. Meanwhile, Electricité de France, which operates the reactors, has ordered immediate checks at all its nuclear generator sites.

**Peter Coles**

## OPTICAL FIBRE LINKS

### Pacific cable breaks

#### Tokyo

THE new trans-Pacific optical-fibre cable (TPC-3) linking Japan and the United States has suffered a serious break in transmission only months after it began operations.

TPC-3 is the first optical-fibre link between the United States and Japan and began leased-line and telephone services in April. But on 18 July the line broke down and transmission was not restored until 22 August. The breakdown, which has not been reported in Japan, caused problems for workers at Tokyo University who are planning to use the cable for a computer link with the University of Hawaii (see above). Instead, the university has been using satellite transmission.

The break was traced to a point 4,500 km from Hawaii at a depth of about 5,000 metres. American Telephone and Telegraph (AT & T), which jointly operates the cable with Kokusai Denshin Denwa (KDD), replaced 60 km of cable and a repeater, according to Shigeru Minatani of KDD. The cause of the break is suspected to be a faulty repeater.

**David Swinbanks**



## GREENHOUSE EFFECT

# Looking at cirrus clouds

## Munich

A UNIQUE high-altitude aeroplane will take off in West Germany on 17 September as part of a European airborne team that will investigate cirrus clouds and their effects on the climate. The clouds, which are made up of ice needles and play an important but little investigated role in



Stratolab will look at Cirrus clouds.

global warming, will be studied in detail by the project, called the International Cirrus Experiment (ICE).

ICE, which is funded by four European governments — West Germany, Britain, France and Sweden — as well as the European Communities Commission, will involve simultaneous, parallel flights by up to five aeroplanes above, within and below the cirrus clouds 10 km above the North Sea. Equipment on the planes and on ships below will monitor the relationship between the ice crystals that make up the clouds and the clouds' ability to transmit heat or light. ICE is also expected to deliver important data on the physical properties of the crystals themselves, including their density and structure.

West German project leader Ehrhard Raschke of the University of Cologne says that ICE will complement the data about cirrus clouds obtained by the US-backed FIRE project (First International Radiation Experiment) of 1986. More aircraft will be used for ICE than for FIRE.

In order to maintain a stable climate, the Earth must return most of the Sun's radiation to space. Like other clouds, cirrus clouds allow sunlight to pass through, and when the same energy is re-radiated from the Earth's surface in the form of heat, they reflect it back to Earth. Because of the sparsity of measurements on cirrus clouds, they have remained something of an unknown quantity, but they are thought to play a potentially significant role in the greenhouse effect. The planes will fly up to three times a week for up to five weeks, although Raschke says he will be happy if six successful flights are made. The North Sea was chosen both because the researchers

could obtain the necessary clearance from military and civilian authorities to fly and because it provides a homogeneous background against which to measure heat transfer. Project leaders also believe that the chances are good of finding cirrus there.

The high-flying plane, called Stratolab, is the first turboprop to reach altitudes of 53,000 feet (16 km). Originally called Egret, it is the product of a collaboration between the West German sailplane manufacturer Grob-Werke GmbH, the Texas-based electronics company E-Systems, and the private WIB-Berlin Space Institute. Stratolab was built to fly above 90 per cent of the atmosphere, into the middle of the protective stratosphere ozone layer there. It is the only European research plane that can fly so high.

Stratolab is effectively a sailplane equipped with an engine. At high altitudes, the plane can glide 40 to 45 metres for every metre of altitude lost. The propeller functions in the thin air at only 10 per cent efficiency, so gliding is a necessity. The plane could in theory carry two people, a pilot and a scientist, but in ICE there will

## ACID RAIN

# China blamed for high pH

## Tokyo

JAPAN is suffering from acid rain and China may be one of the sources, according to the results of a five-year nationwide survey released recently by the Environment Agency.

The first suspicions of acid-rain pollution in Japan emerged more than three years ago when researchers reported low pH levels in rain water and some damage to trees (see *Nature* 319, 711; 1986). The agency survey at 29 locations around the country shows that acid rain (defined as rain water with a pH of less than 5.6) is falling throughout the country. But the average level of acidity at the 29 locations (pH of 4.4–5.5) is not as severe as in Europe and North America (average levels about pH 4.0). And the agency has not found any conclusive evidence of damage to trees, lakes or soil by acid rain. But it fears that damage may result even at these levels over the long term.

Japan implemented strict measures to combat air pollution in the early 1970s and as a result, emissions of sulphur and nitrogen oxides that cause acid rain have been kept down. But there is growing evidence that the continental mainland, in particular China, may be a source of airborne pollution, and with the rapid industrialization of China the problem is likely to get worse.

In 1986, the Environment Agency reported rising levels of a pesticide

## LITHUANIA

# University reopens

## London

THE 'Vytautas the Great' University of Kaunas is to reopen on 1 September, after a gap of nearly 40 years, in response to a public campaign in Lithuania.

The university was founded in 1222, at a time when Kaunas was the capital of the newly independent state of Lithuania. The university was closed down in the Second World War during the Nazi occupation, and briefly reopened under Soviet rule, but was then suppressed in 1950 as part of Stalin's clampdown in the Baltic republics. Although the university was replaced by a medical school and a polytechnic institute, the closure effectively deprived Kaunas of university-level education in the humanities and the natural sciences.

Vera Rich

be no passenger because the cabin is not pressurized. The pilot has to wear a specially fitted space suit. The Berlin institute, run by West German astronaut Reinhard Furrer, is currently working on equipment to measure stratospheric ozone and other trace gases using the plane.

Steven Dickman

(benzene hexachloride) that is not used in Japan in a lake in the northern island of Hokkaido. And the agency suspects that the pesticide, which is used in China and Korea, may have been blown over from the continent (see *Nature* 320, 478; 1986). Later the same year, the Shimane Prefecture Sanitation and Pollution Research Institute which faces the Japan Sea reported that strongly acidic rain (with pH as low as 3.1) frequently falls in the area. As there are no factories nearby, the continental mainland, in particular China, is again suspected. And the results of the present survey show that levels of sulphur and nitrogen oxides in acid rain on the Japan Sea coast increase in winter when prevailing winds blow from the continent.

China suffers from severe acid rain problems and during a recent visit to China by Japan's former prime minister Noboru Takeshita, Japan, at China's request, allocated ¥10,000 million (\$70 million) in overseas development aid to build an environmental institute in Beijing that will have pollution monitoring stations around the country. The institute will carry out research and train state and municipal officials to deal with pollution problems. But as a result of the Tiananmen Square massacre, the project has been temporarily suspended. Hisakazu Kato of the Environment Agency, however, expects that the project will pick up again in "the very near future".

David Swinbanks



## Ethical dilemmas

SIR—David G. Potter (*Nature* **340**, 180; 1989) says “inescapable ethical dilemmas” exist in animal research and that “*Nature* can no more escape the moral responsibility when it publishes research involving animals than can be investigated”.

It is interesting that Potter does not discuss the ethical dilemma of scientists being able to cure human disease, including mental disorders, by animal research, but being unable to do so because animal research has been outlawed. Is it not cruel for humans to suffer pain from diseases that could be curable by animal research? Nor does he discuss his moral responsibility to deny himself all medical care from previous animal research because he wishes to deny future generations the benefits of longer lifespans by future animal research, which Potter wishes to end.

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## DNA fingerprinting

SIR—Do science and the law “differ in temperament”<sup>1,2</sup> to such an extent that evidence recognized as “true” by one would not be recognized as such by the other? Surely any difference is rather that a greater degree of certainty is required when individual freedom, and perhaps in some cases life, is at stake, than when clues for further experimentation are being sought. If Eric Lander’s advice that expert guidelines should be established for DNA fingerprinting<sup>3</sup> is taken, it is to be hoped that science and the law can achieve a consensus on what constitutes acceptable evidence.

There are two major uncertainties in any ‘DNA fingerprinting’ test — the reliability of the probe data with respect to the sample population and the reproducibility of data from a particular laboratory. The first problem could perhaps be addressed in a similar way to the identity parade, that is, suspects and ‘controls’ from a similar genetic background could be analysed in parallel. The problem of laboratory quality control is not unique to the forensic service and can be dealt with by having samples coded before analysis and by the inclusion of known samples (also coded) in each test. Statistical analysis of any laboratory’s output would then be possible, enabling a continuous assessment to be made of the quality of its results.

On a practical note, variation of mobility of DNA fragments in gels is generally

less of a problem in polyacrylamide gels, and the recent description of CA repeat-length polymorphisms<sup>4,5</sup> offers a new set of markers that could be used for fingerprinting<sup>6</sup>. Direct sequencing of amplified CA repeats would provide a very rigorous internal control for repeat-length polymorphisms.

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## Misquoted

SIR—Commenting on the article “The case of the peripatetic fossil” (*Nature* **338**, 613; 1989), K. S. Jayaraman (*Nature* **338**, 694; 1989) said: “Gupta’s colleagues at the Centre for Advanced Study in Geology, including its director, Dr A. K. Prasad, described Talent’s allegations as ‘a conspiracy to denigrate a top Indian scientist’”. This statement is misleading: Jayaraman did not consult us or other colleagues on this issue. As geoscientists working in the same institution, we are directly and deeply concerned with all aspects of the controversy and are alive to the need to find the truth.

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## Raman’s prize

SIR—The question of whether Raman deserved an undivided Nobel prize for his 1928 discovery of the light-scattering effect and whether Mandelstam’s achievements in this area have been ignored by the Nobel committee still provokes some controversy<sup>1</sup>. But although it is true that some landmark discoveries made by Soviet scientists in this century have failed to receive adequate Nobel recognition, the selection of Raman over the claims of Mandelstam for the 1930 Nobel prize in physics is not a tainted one.

After attending the Sixth Congress of Russian Physicists, C. G. Darwin reported in *Nature* that Mandelstam and Landsberg “had independently discovered Raman’s phenomenon, the scattering of light with changed frequency”<sup>2</sup>. Raman refuted this claim emphatically, by writing: “The Russian physicists, to whose observation on the effect in quartz Professor Darwin

refers, made their first communication on the subject after the publication of the notes in *Nature* of 31 March and 21 April. Their paper appeared in print after sixteen other printed papers on the effects, by various authors, had appeared in recognised scientific journals<sup>3</sup>.” This has not been repudiated by Raman’s competitors.

According to the published census of Nobel nominees and nominators<sup>4</sup>, for the 1929 Nobel prize, Raman received two nominations from N. Bohr and C. Fabry, while Mandelstam received none. In 1930, Raman received ten nominations, which included those of N. Bohr, E. Rutherford, C. T. R. Wilson and L. de Broglie (all previous Nobelists). In the same year, Mandelstam received two nominations and Landsberg one.

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## Time-reversal

SIR—Would not one possible solution to the dilemma posed by Huw Price in “A point on the arrow of time” (*Nature* **340**, 181; 1989) be found in Guth’s inflationary period? According to that, the Universe does begin in a highly disordered state at Time 0 but at a very early stage inflation sets in and establishes a condition of low entropy, at that point. We thereby have a disorder-disorder type of universe and Hawking has no need to find means of excluding it.

To speak of “temporality” or “atemporality” becomes a problem in semantics and not in physics, and time’s “asymmetry” is no longer consequent on initial conditions but only on a subsequent inflation.

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SIR—Huw Price’s philosophical discussion of time-reversal in a contracting Universe echoes the discussion put forward by physicist Thomas Gold at least 25 years ago, at a Cornell conference. A more ‘popular’ account later appeared in *The Runaway Universe*, by Paul Davies (Dent, 1978). Philosophers, it seems, still underestimate physicists as much as the latter underestimate the former. Or does time run more slowly in Australia, so that news of these developments has not yet reached Sydney?

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# Neptune's satellites predicted?

A theory believed to be successful in predicting the satellites of Uranus is not widely accepted. Much will hang on whether it has been successfully applied to Neptune.

Last week's encounter of Voyager 2 with Neptune will be, when the data have been analysed, a fateful occasion for Dr Andrew Prentice of Monash University in Melbourne. For the past several years, Prentice (a mathematician by trade) has had some success in predicting the properties of the outer planets and their satellites, most conspicuously at the encounter between Voyager 2 and Uranus in 1986. By definition, the encounter with Neptune will have been the last chance to test the accuracy of his prediction — there are no more planets left. But it is unlikely that the argument surrounding Prentice's work, which raises absorbing questions about the validity of predictive theories whose mechanisms are disputed, will be stilled as quickly.

Prentice himself makes no secret of the origin of his theory, which is based on the attempt by Laplace, in the eighteenth century, to relate the radii of the orbits of the planets to each other. Indeed, Laplace's theory was an important part of his great work *Exposition du Système du Monde* by which he is most widely remembered. For the period, the insight is quite remarkable. It is no shame to Laplace that he was unable to describe a mechanism whereby rings of gas and dust could condense into planets.

That is what Prentice reckons to have done, in the process reaching conclusions about the way in which the radii of the satellites of the multi-satellited planets beginning with Jupiter are related to each other. For the regular satellite systems such as those of Uranus, in which the satellites revolve in the same direction as the rotation of the planet, there is held to be a constant ratio between the radii of successive planets. This is the prediction that worked spectacularly well for Uranus. But Neptune is different in at least one respect: Triton, the largest of its satellites, travels in an orbit inclined to the ecliptic and to the plane of Neptune's equator, and in a direction opposite to that of the planet's own rotation. Few dispute Prentice's assumption that Triton was formed separately, but probably from the same material as Neptune (and, for that matter, Pluto). Part of his present interest is in the radii of the other pro-grade satellites of Neptune. Another is in the chemical composition (and thus the mean density) of objects such as Triton, as calculated from the supposed history of the condensing gas cloud.

The argument about Prentice's "modern laplacian theory" centres not on the predictions, but about the mechanisms it supposes to control condensation. The driving force both for the shedding of successive planetary rings and the formation of the separate satellite systems is supposed to be turbulence caused and then sustained by convection, presumably from the evolving primitive Sun. If there is such a mechanism, and if it can be sustained, there is no doubt that the consequences for a contracting solar nebula would be profound. In the outer regions of the cloud, for example, turbulent velocities would be supersonic, while turbulent stress would enormously augment the normal pressure of the gas. People's quarrel with Prentice hangs most simply on the circumstance that turbulence is a dissipative process whose effect would be to heat the gases involved in the turbulent motions (as might be expected for convection).

So what will people say if Prentice's predictions for the satellites of Neptune are shown to be as accurate as those for the satellites of Uranus in 1986? There will, of course, be two opinions. Prentice, his associates and his supporters will no doubt be confirmed in their opinion that they are on to something. Nobody would expect them to behave differently. But, equally, their critics will continue to insist that, in the absence of a plausible mechanism, the theory has no status.

Where will the truth lie? It is appropriate to recall that, in Laplace's time, strictly empirical theories were respectable.

Dulong and Petit, for example, contemporaries of Laplace, made sense of the specific heats of metals in a manner not made respectable until the arrival of the kinetic theory. Their rule, then a relationship between specific heat and atomic weight, should then, of course, have fallen into disrepute with the discovery that electrical conduction is mediated by electrons, only to be resurrected with the arrival of the quantum theory.

Then, at least, strictly empirical theories were both common and acceptable, as they still are in many fields — the various rules about species diversity with which ecologists are concerned are good examples.

So may it be that Prentice has arrived at an empirical rule for relating the properties of satellite systems to each other, and happens merely to have put forward an

implausible mechanism that will eventually be refined? That, no doubt, is how the critics will be answered if the Neptune predictions are borne out by the observations of Voyager 2. And there will be some (but not Prentice, who does not acknowledge that his mechanism is faulty) who complain that too slavish a concern for mechanisms is the enemy of progress. That argument would have force if the issue were essentially practical, say the prediction of the superconducting properties of various ternary oxides (and, in the absence of an understanding of the mechanism of high-temperature superconductivity, empirical theories have enjoyed a new lease of life in that field during the past two years).

But there are two reasons why the licence does not apply to the prediction of planetary satellite systems, of which the simplest and more trivial is that there are only limited data to account for. With Voyager 2 now past Neptune, and with the prospect that it will be years or even decades before there is detailed information on other planetary systems, further prediction has no practical purpose. The situation is much like that in particle physics, where empirical arithmetical rules relating the masses of known particles to each other are forever popping up, but where the true objective can only be a surer understanding of their physical basis. In that connection, plausibility is essential.

The more telling reason why Prentice's critics will not fall silent is that there is already a great deal of successful planetary science entailing only plausible mechanisms. Indeed, it is one of the achievements of the past few decades that so much has been done to understand the way in which the Solar System formed from primæval gas mixed with stellar debris. On that view, there was indeed a condensing nebula, the variations of chemical composition from one planet to another are intelligible in terms of the condensation temperatures of their constituents, but there must be many features of the Solar System that will be explained only historically, as consequences of past events still unknown. None of that is unrespectable, or a manifestation of orthodoxy conspiring to suppress the heterodox.

Even so, it will be interesting to see whether Prentice's predictions for Neptune are confirmed. **John Maddox**



# Tsunami-wave seismology

Wayne Thatcher

Tsunamis (seismic sea waves) are among the most awesome and destructive effects of great sub-sea earthquakes. They result when fault slippage beneath the sea floor abruptly displaces the ocean bottom and generates a shallow-water gravity wave whose wavelength and speed depend on water depth at the epicentre and along the travel path. In open ocean basins, tsunami wavelengths are several hundred kilometres and their amplitudes are small and imperceptible. But near shore, the decreasing water depth and bottom friction cause wavelengths to decrease while amplitudes increase. The resulting waves, sometimes tens of metres high, have caused destruction and loss of life in coastal regions both near the earthquake source and thousands of kilometres distant (see figure). In new work (*J. geophys. Res.* **94**, 5627–5636; 1989) Kenji Satake shows that the information that can be derived from tsunami-wave arrival patterns can be as useful as conventional solid-earth seismology in determining the character of submarine earthquakes. Indeed, there are some respects in which tsunamis offer advantages over elastic-wave seismology.

Tsunami wave motion is most commonly recorded as changes of shoreline water level measured by tidal gauges, stilling wells set in the water and connected to the outer ocean by a tube or orifice. The recordings produced are in many ways analogous to seismograms and the use of tsunami waves in seismology has paralleled that of elastic waves.

Until about 1970, seismograms were used primarily to determine the arrival times of body and surface waves so as to locate earthquakes and to infer elastic moduli and wave speeds as functions of depth in the Earth along the route between an earthquake and its detector. First-motion directions and wave polarizations were used to constrain the orientations of earthquake fault planes and the direction of fault slippage. More recently, it has become possible to infer from the waveform information contained in seismograms detailed characteristics of earthquake sources and much better mappings of the three-dimensional elastic properties of the Earth's interior. Similarly until very recently, tsunami-wave seismologists were able to use only first-arrival time and initial-motion direction to estimate the spatial extent and direction of vertical displacement of the sea floor. But now it is becoming clear that tsunami recordings contain considerably more information about earthquake sources than had previously been appreciated: elastic-wave

seismologists are watching new developments with interest.

The excitement is a result of the research by Satake, a Japanese seismologist whose work over the past 4 years has culminated in the publication of his new paper showing that tsunami waves carry information about the spatial distribution of earthquake fault slip comparable to that provided by conventional seismograms. Satake estimated the slip distributions in the 1968 Tokachi-Oki ( $M_w = 8.2$ ) and 1983 Japan Sea ( $M_w = 7.8$ ) earthquakes using arrays of 6–12 Japanese tidal gauges located within about 500 kilometres of these

IMAGE  
UNAVAILABLE  
FOR COPYRIGHT  
REASONS

30-foot tsunami waves raised by the 1964 Alaska earthquake caused havoc along the whole North American Pacific coast.

events. He shows that slippage over sub-regions of the earthquake fault plane as small as 30–50 km can be resolved by the regional tsunami waves and that the derived slip distributions resemble those independently obtained from seismic-wave analyses. The good spatial resolution of fault slip is due in part to the excellent distribution of regional tidal gauges in the Japanese islands, which have been densely instrumented since 1950. Elsewhere, and for earlier events in Japan, tsunami-wave inversions must rely largely on distant recordings which, because of their longer-period character, will have poorer spatial resolution of fault slip.

Satake's work has elicited widespread interest because of increasing awareness of the importance of slip heterogeneity and its influence on faulting processes. Heterogeneity creates slip-deficient zones with the occurrence of each great earthquake, and the behaviour of these zones from one cycle of seismic strain release to the next is important from several standpoints. If deficits generated in one cycle are made up by seismic slippage in the next strain release episode, then great earthquakes will differ significantly from cycle to cycle. If, on the other hand, there are even gross similarities from one event

to the next, inter-earthquake aseismic slippage in the deficient zones will be required to match the long-term slip rates.

The relative importance of aseismic slippage, its spatial distribution and temporal variability all bear importantly on the mechanics of subduction and the nature of earthquake-related crustal deformation. Better definition of the regions of high seismic slip ('asperities') will contribute to elucidating their influence on earthquake mechanics (H. Kanamori *A. Rev. Earth planet. Sci.* **14**, 293–322; 1986). To the degree that earthquake slip patterns can be forecast, the expected strong ground-motion effects of recurrent events can also be estimated. Also, although it is thought that fault slippage in one large earthquake may affect the timing of the next comparable event (K. Shimazaki & T. Nakata *Geophys. Res. Lett.* **7**, 279–282; 1980), the influence of slip heterogeneity on earthquake recurrence is not yet well understood.

In this context, important questions about the tsunami-wave method include its typical resolution over the entire rupture plane and its advantages and shortcomings compared with seismic and geodetic techniques of slip estimation. For earthquake rupture zones lying entirely beneath the sea floor (as in the 1968 and 1983 events studied by Satake), tsunami data could elucidate the complete slip distribution, but movements on the deepest portions of the fault plane will be the least well resolved because they generate the smallest seafloor displacements. Even for these events, estimates of slippage at depth might be improved by including constraints from seismic or geodetic data. For subduction zone events whose deepest rupture segments lie below continents or island arcs, such information would be essential to obtain the complete distribution of earthquake slip.

A possible complicating factor in using the tsunami method is uncertainty about the degree to which sea-floor displacements reflect only the elastic deformation resulting directly from fault slip. It is widely recognized that some great tsunamis, such as those caused by the 1946 Aleutian earthquake ( $M = 7.4$ ) and the 1896 earthquake at Sanriku, Japan ( $M = 7.9$ ) were mostly the result of large-scale slumping of loosely consolidated sediments lying on the trench-arc slope. These movements are interesting in their own right and can be studied using tsunami-wave inversion methods, but as far as the estimation of seismic slip is concerned, they represent contaminating noise. Although these great earthquake-induced slumps are rare, it is not yet clear whether smaller events of similar type are more common and if so whether they contribute significantly to tsunami

waveforms generated by large earthquakes.

Despite these limitations, tsunami-wave methods have several advantages in the estimation of fault slip. Because of their low propagation velocities (around  $0.2 \text{ km s}^{-1}$ ), tsunami waves generated from different segments of a great earthquake rupture will typically arrive at tidal gauge stations separated sufficiently to be readily distinguished. And because the period of tsunami waves (5–20 minutes) is much longer than the duration of rupture in even the largest earthquakes (several minutes), the final static fault slip distribution can be recovered from the data. Seismological methods of slip estimation are much cruder. Long-period bodywaves can provide qualitative mappings of regions of high slip (for example, see S.L. Beck & L.J. Ruff *J. geophys. Res.* **92**, 14123–14138; 1987), but because the seismograms are dominated by the effects of slip acceleration and deceleration in the few regions where strain release is highest, the distribution and magnitude of slippage elsewhere on the rupture is poorly determined.

Furthermore, seismic wave periods are not long enough to define the full static offsets in even the high-slip zones and, because lateral variations in the Earth's structure are imperfectly known, unavoidable uncertainties are introduced into waveforms corrected for source–receiver

transmission effects. In contrast, tsunami propagation depends mostly on ocean depth, and supercomputers in use now are powerful enough to permit tsunami waveforms to be accurately corrected for the known effects of ocean bathymetry. Although land-based geodetic survey measurements of earthquake deformation are free of these shortcomings, they can constrain slippage only on the onshore or near-shore segments of the fault, typically less than a third of the entire rupture zone.

Given these advantages and tidal gauge records dating back to the mid-nineteenth century, the scope for new research is very broad. Sub-sea slip distributions can be estimated for many more circum-Pacific great earthquakes, some of which occurred before 1904, when modern damped seismographs were first deployed world wide. As a result, much more can be learned about the source characteristics of older earthquakes of considerable importance to seismology. For more recent events, further comparisons between tsunami-wave estimates of earthquake slip and those obtained by geodetic and seismic methods will better illuminate the advantages and shortcomings of each technique. □

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## GALACTIC EVOLUTION

# Starbursts, quasars and all that

*Cedric Lacey*

STARBURST galaxies, as the name implies, are the sites of abnormally rapid star formation. In the case of starburst nuclei, the most luminous examples, this activity occurs at the heart of galaxies that are otherwise normal and so have already converted most of their gas into stars<sup>1</sup>. On page 687 of this issue<sup>2</sup>, Hernquist argues that the vast quantities of fresh gas needed to fuel a starburst can be driven into the galactic centre by the tidal interactions that accompany the merging of two galaxies. It may even be that starbursts, Seyfert galaxies and quasars are all part of a simple evolutionary sequence triggered by such mergers.

The activity of nuclear starbursts is confined to the central 1 kiloparsec of a galaxy, itself perhaps tens of kiloparsecs in diameter. The most dramatic examples are  $10^{11}$ – $10^{12}$  times as luminous as the Sun. Most of the radiation is emitted in the far-infrared — the optical and ultraviolet emission from the massive young stars being absorbed and re-radiated at longer wavelengths by a thick layer of dust that shrouds starbursts. The stars must form at rates exceeding 10–100 solar masses ( $M_{\odot}$ ) a year to produce such luminosity even if, as the evidence<sup>3</sup> seems to indicate, only

massive bright stars are formed. For comparison, the rate of formation of stars in our entire Galaxy is about  $3 M_{\odot}$  a year.

Starburst nuclei are also very rich in gas, containing perhaps  $10^9$ – $10^{10} M_{\odot}$  of molecular hydrogen. Nevertheless, a starburst could consume this amount of gas in  $10^8$ – $10^9$  years, a small fraction of the lifetime of a galaxy, the brightest starbursts being the shortest lived<sup>4</sup>. It is reasonable to infer that the gas is delivered to the galactic centre over a similar period, so that the influx is extremely large.

An interesting comparison can be made with the fuelling of active galactic nuclei — bright, compact sources of non-thermal continuum radiation, some of which is reprocessed by dust into thermal continuum radiation or by ionized gas into emission lines. The source is believed to be a massive black hole ( $10^8$ – $10^9 M_{\odot}$ ) that is accreting gas — at a rate of  $10^{-3}$ – $1 M_{\odot}$  a year for Seyfert galaxies, which are as luminous as starburst nuclei, or up to a hundred times faster for quasars, which can be brighter still. Energy is released much more efficiently by the gravitational heating close to a black hole than by nuclear burning in a starburst, which is why active nuclei can be as luminous as star-

bursts, despite the smaller fuelling rates.

The source in an active nucleus is less than 1 parsec across, so that the gas must be fed into a much smaller volume than in starburst nuclei. It turns out that many of the brightest starbursts contain a Seyfert nucleus which emits a substantial fraction of the total energy, indicating that the gas-supply mechanisms in the two systems are somehow connected. Observations suggest that fuelling mechanisms are related to large-scale galactic dynamics: the brightest starburst galaxies virtually all show evidence of interacting tidally or merging with other galaxies<sup>5</sup>; and both Seyfert and starburst activity are often associated with the presence of a stellar bar<sup>6</sup> (an oval-shaped enhancement in the surface density, in the centre of the disk, which rotates like a rigid body).

This is the starting point for Hernquist<sup>2</sup>, who has modelled the effects of a merger between a parent galaxy like the Milky Way, containing stars and gas in a disk, and a smaller, satellite galaxy that contains stars only. Initially, the satellite is orbiting at the edge of the disk, but it spirals in as it loses energy and angular momentum by dynamical friction against the parent's stars and gas. The stars end up gaining energy and angular momentum, but the gas ends up losing both, the reason for the difference being the dissipative nature of the gas. The gravitational field of the satellite perturbs the gas orbits from their previously circular form, causing gas streams to intersect and shock, building up regions of high density. Under the influence of this gravitational field, some of the high-density gas collects into a massive, gravitationally bound cloud, which sinks to the galactic centre by dynamical friction against the stars. Meanwhile, the satellite galaxy is tidally disrupted. The net result is that about a third of the gas initially in the disk — around  $2 \times 10^9 M_{\odot}$  — is deposited into a central region less than 400 parsecs in radius. The entire merger takes about  $10^9$  years, but the gas cloud, once formed, sinks to the centre in only  $10^8$  years.

The simulations thus indicate a plausible mechanism for rapidly injecting large quantities of gas into the central regions of galaxies, but they do not answer the question of what happens to the gas once it gets there: they do not include any treatment of star formation, and the spatial resolution is limited to around 400 parsecs. It is certainly plausible that the large gas density built up at the galactic centre should result in vigorous star formation, owing to the reduced timescales for cloud–cloud collisions and gravitational instability and fragmentation<sup>7</sup>, but there is no reliable quantitative theory for this. Once stars are being formed, the subsequent supernova explosions of dying massive stars will heat the gas by driving shock waves into it. Neglect of this effect is the



most serious deficiency of the simulations, because it could greatly inhibit the processes tending to concentrate the gas into the centre.

Because of their limited resolution, the simulations provide information neither on how a central black hole might be formed, nor on how an already existing black hole might be supplied with gas. There have been various proposals for the latter process. Norman and Scoville<sup>8</sup> have suggested that gas drag or dynamical friction causes massive gas clouds to spiral down to a 10-parsec radius, where the gas forms a dense star cluster. Gas shed by stars in this cluster as they die is assumed to form and fuel the black hole. An alternative picture<sup>9</sup> is that a bar instability develops in the central gas disk once it becomes sufficiently massive. The bar loses angular momentum by gravitational torques, again carrying the gas down to a small volume, after which turbulent viscosity sets in, driving accretion onto the black hole.

As the rate of star formation decays with the consumption or dispersal of the gas and dust surrounding the central source, the external appearance of the central region will change: at first, the

light from young stars, re-radiated by dust, will be seen; then there will be thermal emission from dust and non-thermal continuum from the active nucleus, both in the infrared; finally, with dust removed, optical emission from the active nucleus could become apparent if there is still sufficient accretion onto the black hole. Thus, one may have the evolutionary sequence of starburst → Seyfert → classical quasar. If all that is needed to trigger this sequence in a spiral galaxy is for it to have a moderately sized companion, many spiral galaxies may have gone through such a phase at some time. □

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## MARROW TRANSPLANTATION

# Can cord blood be used?

David C. Linch and Leslie Brent

TRANSPLANTATION of HLA-matched bone marrow is currently the treatment of choice for selected patients with aplastic anaemia, leukaemia and inherited disorders of the bone marrow. The availability of HLA-identical sibling donors limits this option to roughly a quarter of otherwise suitable patients, so that panels of HLA-typed individuals willing to donate bone marrow have been set up in many countries. The panels need to be very large — a pool of 250,000 donors is estimated to have a 59 per cent chance of providing a compatible donor for any one patient, and with increasing pool size there is a disproportionately smaller increase in the number of successful matches<sup>1</sup>. In a recent report, Broxmeyer *et al.*<sup>2</sup> make the intriguing suggestion that the neonatal blood retained in the placenta contains sufficient blood stem cells to serve as a transplant inoculum. Neonatal blood could be collected, largely from the umbilical cord, and cryopreserved to build up a large bank, thus overcoming some of the difficulties associated with volunteer panels. The logistical problems associated with the creation and maintenance of such a bank are, however, quite formidable.

It was shown a quarter of a century ago that the blood of fetal mice contains large numbers of haemopoietic stem cells detectable in spleen colony-forming assays<sup>3</sup>.

Likewise, second-trimester human fetal blood has a high incidence of haemopoietic progenitor cells estimated by *in vitro* colony-forming assays<sup>4</sup>; although the frequency is known to decline during the course of gestation, umbilical cord blood contains a higher number of progenitor cells per unit volume than adult blood, and it is roughly equivalent, in this respect, to adult bone marrow. This is amply confirmed by Broxmeyer *et al.* in over 100 cord blood samples. It is an article of faith that when there are large numbers of haemopoietic stem cells there will be large numbers of transplantable stem cells, but this is a reasonable assumption.

The novel data reported by Broxmeyer *et al.* concern the volume of cord blood that may be extracted: over 50 ml on average, but nearly 200 ml using an "optimized" method involving removal of blood from the maternal end of the transected cord whilst the placenta is still *in utero*, to which is sometimes added blood obtained by needle aspiration of engorged vessels on the fetal surface after expulsion of the placenta. This figure is surprisingly high — previous estimates<sup>5</sup> of fetal blood volume in the placental vessels immediately after birth have been of the order of 75–125 ml.

A 100-ml sample of cord blood can be expected to contain about  $2 \times 10^6$  myeloid

(GM-CFC) progenitor cells and  $1 \times 10^6$  erythroid progenitor cells, which should be sufficient for reconstitution after allogeneic transplantation<sup>6</sup> provided that the stem-cell/progenitor-cell ratio is not appreciably less than in adult bone marrow. Only clinical studies can prove this point.

There are, however, two possible difficulties with the strategy proposed by Broxmeyer *et al.*, both relating to the possible development of graft-versus-host (GVH) disease. First, it is common practice when transplanting matched unrelated bone marrow to deplete it of mature T lymphocytes in order to minimize the GVH response; we are, however, told by Broxmeyer *et al.* that any fractionation of neonatal blood results in unacceptable losses of progenitor cells. This could be surmountable.

More worrying is the second possibility — that neonatal blood is contaminated by maternal cells. Some degree of leakage from fetus/neonate to the mother occurs in up to 50 per cent of births, usually during parturition, and occasionally clinically significant leakages occur from mother to fetus<sup>6</sup>. The incidence of relatively minor leakages from the maternal to the fetal circulation during parturition is not known, but it could be high. The magnitude of maternal blood contamination could be increased by the vigorous venesection from placental vessels before and after delivery of the placenta that seems to be required for the collection of optimal amounts of blood. Although adult blood possesses relatively few stem cells, the transfer of mature T lymphocytes incompletely matched with the histocompatibility antigens of the recipient could contribute unacceptably to GVH disease. Neonatal blood is virtually as immunocompetent as adult blood so far as the generation of cytotoxic T lymphocytes is concerned<sup>7</sup> and therefore has the potential for inducing GVH disease.

Finally, Broxmeyer *et al.* suggest that cryopreserved cord blood cells could be used for autologous bone marrow transplantation. The prospect of selected individuals (presumably the babies of the wealthy) having their cord blood stored as an insurance against the ravages of leukaemia or nuclear accident in adult life is more than a little disturbing. □

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## HIGH-ENERGY PHYSICS

# Beaming in on the $Z^0$ particle

David J. Miller

WITH four particle accelerators now available with sufficient energy to produce the  $Z^0$ , one of the most interesting particles in fundamental physics, many exciting results can be expected. Preliminary data from two of these accelerators, the Fermilab 'Tevatron' proton-antiproton collider<sup>1</sup> and the Stanford Linear Collider<sup>2</sup> (using electrons and positrons), are published in the 14 August *Physical Review Letters*. These data have been used to measure the  $Z^0$  mass and the width of its excitation curve (how the production rate varies as a function of the particle's mass; this is linked to its lifetime by the uncertainty principle). These are among the most fundamental of the many parameters in the standard model of elementary forces.

The  $Z^0$  mass is directly linked to the way in which the weak force 'mixes' with the electromagnetic force in electroweak theory. Its width is determined by the strength of its couplings to all possible pairs of particles into which it can decay: these include leptons (electrons,  $e^\pm$ , muons,  $\mu^\pm$ , or taus,  $\tau^\pm$ , all of which are relatively easy to recognize) or quark-antiquark pairs which produce jets of strongly interacting particles (protons, pions and so on). The new data are not yet sufficiently precise to make definitive tests of the standard model, but they show how rapidly the measurements are improving.

Because of the large width and short lifetime of the  $Z^0$ , its presence can be detected only by measuring its decay products. At Fermilab, researchers have recorded 132 muonic events and 64 electronic ones. (Decays to strongly interacting particles are masked by competing events in which these are produced directly in the proton-antiproton collisions.) The mass of each  $Z^0$  has to be reconstructed from the directions and energies of the two muons or electrons from the decay. From the distribution of these masses the authors find the average value of the  $Z^0$  mass to be  $90.9 \pm 0.3$  GeV, and the resonance width,  $3.8 \pm 0.8$  (statistical)  $\pm 1.0$  (systematic) GeV.

The Stanford Mark II detector can identify strongly interacting particles from  $Z^0$  decays as well as leptons, and has detected 94 pairs of these, together with 12 muon and tau pairs<sup>2</sup>. The Stanford researchers scan the collision energy through the resonance zone, and count the rate of  $Z^0$  production. These data have to be normalized, achieved by counting the rate of small-angle electron-positron deflections which occur electromagnetically. The resulting  $Z^0$  mass is  $91.11 \pm 0.23$  GeV, with a width of  $1.61^{+0.6}_{-0.43}$  GeV.

These results are much more precise

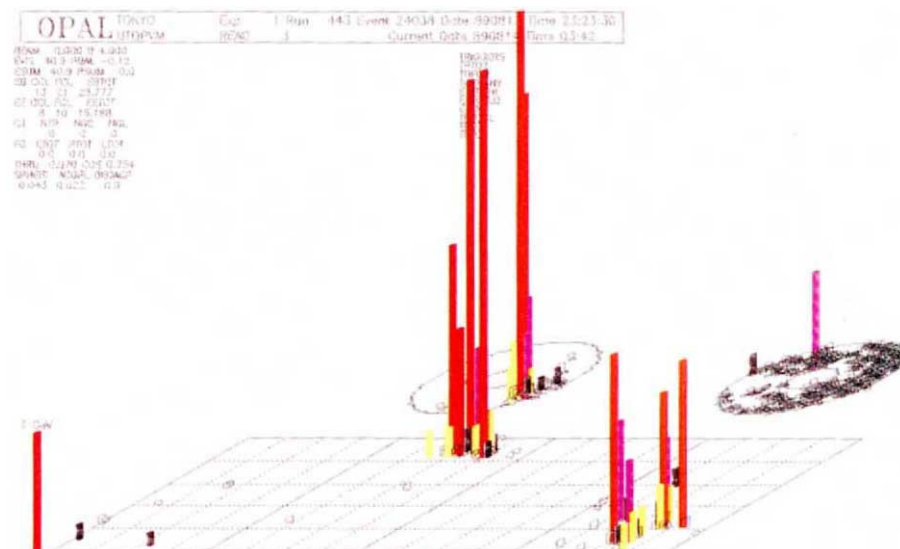
than previous values, obtained at the CERN SPS proton-antiproton collider. The published Stanford width is particularly interesting because it is more than one standard deviation less than predicted by the standard model of electroweak interactions. Since the paper was submitted for publication, a further 140  $Z^0$ s have been detected. Preliminary analysis of these, reported at a recent meeting\*, suggests that the resonance is in fact a little wider, but it is still more than one standard deviation less than the predicted value.

If borne out by further measurements, the reduced width would deliver the first blow in 20 years to the standard model. One modification to the model would be to allow for additional, heavier  $Z^0$ s that share the weak neutral coupling with the standard  $Z^0$ ; these would reduce the width

of the standard particle's resonance. Instead of making the  $Z^0$  width the variable in fitting the measured resonance curve, the Stanford group also uses an alternative approach in which the standard-model value of the width is assumed and the number of  $Z^0$  decays to neutrino-antineutrino pairs is determined. These decays are invisible, but still contribute to the shape of the resonance. The Stanford group finds the number of light neutrino types to be  $3.8 \pm 1.4$ , on the assumption that each kind has the same coupling as the well known electron and muon neutrinos.

The number of neutrino types is directly linked to the thermodynamics of nucleosynthesis during the Big Bang, determining the primaevial abundance of helium in the Universe. We believe there are at least three types of neutrinos (the partners to electrons, muons and taus). The new result would allow two more types — not yet sufficiently precise to constrain the helium abundance to within astrophysical

## Early $Z^0$ s encourage CERN physicists



Following a one-week trial run, the Large Electron-Positron collider (LEP) at CERN is the fourth accelerator to produce  $Z^0$  bosons, the first being the Super Proton Synchrotron at CERN, with Stanford and Fermilab succeeding recently.  $Z^0$ s have been detected in all four of LEP's underground experiments, although inevitable teething problems occurred on the accelerator, first commissioned little over a month ago.

The figure shows a 'lego' plot (courtesy of Tokyo University) of one of the first  $Z^0$ s to be detected at LEP, by the OPAL collaboration. The graph, which earned its title from the original method of photographing stacked Lego bricks, depicts the energy flow recorded in each of the lead glass blocks in a 'calorimeter' surrounding the interaction zone at OPAL. The calorimeter responds to electromagnetic showers caused by electrons or  $\gamma$ -rays. In this event,

most of the signal probably came from  $\gamma$ -rays due to the decays of strongly interacting particles in the two narrow jets from the decay of  $Z^0$  to a quark-antiquark pair.

In the plot, the cylindrical walls of the barrel-shaped calorimeter are rolled out flat with the end caps shown separately; the height of each bar is proportional to the energy deposited in a glass block.

The preliminary experimental run temporarily interrupted the commissioning programme for LEP, which has now been resumed. Among difficulties encountered during the run was an unexpected tendency for the beams to spread out vertically as a function of momentum (dispersion) even though there are no vertical bends in the machine. Nevertheless, physicists at CERN are greatly encouraged to have detected so many  $Z^0$ s — 50 in all — at such an early stage in its programme. □

\* Lepton-photon symposium, Stanford, 5–11 August 1989.



limits — but it is getting closer.

If the width does turn out to be compatible with the standard model, the race will be on between Stanford and the new Large Electron-Positron collider (LEP; see box) at CERN to determine the  $Z^0$  mass with a precision better than 50 MeV. As described by John Ellis in his recent review article<sup>3</sup>, with this kind of resolution, it should be possible to constrain tightly predictions of the mass of the top quark, the second quark — as yet unobserved — in the heaviest known generation. What is more, if the top quark can be

observed at Fermilab, which generates collisions of the highest energy currently available, its mass together with that of the  $Z^0$  could be used to predict the mass of the Higgs boson, first postulated nearly 30 years ago to explain the masses of all particles in ordinary matter<sup>4</sup>.

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## G PROTEINS

# Gasp: not just another oncogene

Frank McCormick

THE pathways that lead from the activation of cell-surface receptors to cell division remain obscure. Many of the stepping stones along these pathways are proteins encoded by proto-oncogenes: mutations in these genes result in proteins that cause uncontrolled growth. So far, we have not been able to connect the biochemical properties of these proteins and cannot fully describe how any one of them works. On page 692 of this issue<sup>1</sup>, however, Henry Bourne and co-workers describe a putative oncogene that lies on a well trodden path connecting a surface receptor to a familiar second messenger with well documented physiological effects.

The product of this gene is a mutant version of  $G_{as}$ , the GTP-binding subunit of the G protein  $G_i$  which normally transmits signals from the  $\beta$ -adrenergic receptor to adenylyl cyclase, thus causing synthesis and accumulation of the second messenger, cyclic AMP. The possibility that  $G_{as}$  could be an oncoprotein had been anticipated by Bourne in a previous News and Views article<sup>2</sup>. Commenting on a report that a subset of pituitary tumours contains elevated cAMP levels and increased rates of growth-hormone secretion, Bourne proposed that these effects were due to constitutive activation of  $G_{as}$ . Furthermore, cAMP was known to promote growth of pituitary cells, hence the suggestion that activation of  $G_{as}$  could also lead to uncontrolled proliferation.

Bourne and co-workers now report<sup>1</sup> that in pituitary tumours with elevated cAMP levels and growth-hormone production,  $G_{as}$  is activated by somatic point mutations. These mutations are in one of two amino acids: Arg 201, known to be a site

of ADP-ribosylation by cholera toxin, and Gln 227, which is equivalent to Gln 61 of the *ras*-gene-encoded p21 proteins (Fig. 1). Both mutations (like ADP-ribosylation by cholera toxin) destroy the intrinsic GTPase activity of  $G_{as}$ , so that the protein remains in its active, GTP-bound state

The regions of p21 that surround the bound GTP (including the Gln 61 region) have highly conserved counterparts in  $G_{as}$  and all other known G proteins. It is therefore likely that all their GTP-binding sites will have very similar structures. However, *ras* p21 does not have an obvious counterpart to Arg 201 of  $G_{as}$ . What, then, is the role of this amino acid in  $G_{as}$  GTPase activity? Inspection of the sequence of  $G_{as}$  around position 201 reveals two interesting homologies, one with p21, the other with GAP, each suggesting different roles for arginine 201.

The region of p21 that resembles amino acids 189–203 of  $G_{as}$  is the well-known effector-binding site<sup>4,5</sup>. Certain mutations in this region (amino acids 32–40) knock out biological activity of p21 without affecting nucleotide binding or membrane localization. It is therefore considered a likely site for binding of an essential target molecule (hence the name). Most mutations that destroy biological activity also destroy interaction with GAP, indicating that GAP is itself an effector of p21 action<sup>6</sup>. Another interesting feature of this region, revealed by the crystal structure

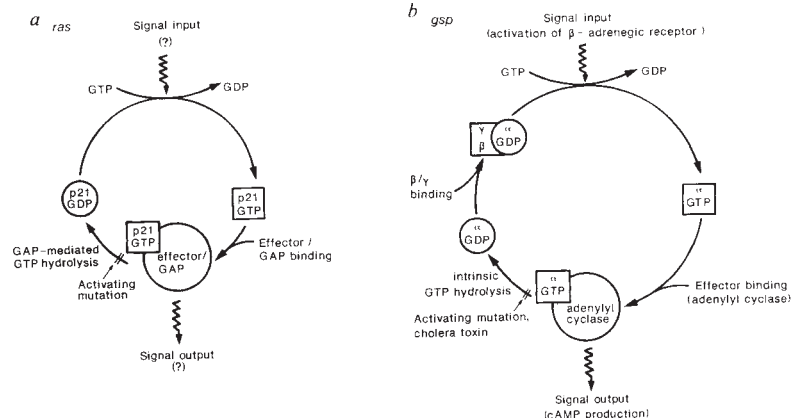


FIG. 2 Roles played by the p21(a) and  $G_{as}$  (b) GTP-binding proteins in signal transduction.

constitutively (Fig. 2). The activated form of  $G_{as}$  is referred to as Gsp (pronounced gasp).

These results are reminiscent of the events that convert *ras* genes to oncogenes, and, as Bourne and colleagues point out, there are interesting comparisons between the two systems. By contrast with  $G_{as}$ , p21 proteins have very little intrinsic GTPase activity. They depend on a second protein, GTPase activating protein (GAP), to convert bound GTP to GDP. Mutations of p21 commonly occurring in human tumours (amino acids 12, 13, 61) prevent GAP from performing this function.  $G_{as}$ , on the other hand, has 'built-in' GAP activity, which confers relatively high GTPase activity. This activity is likely to come (directly or indirectly) from the Arg 201 region of  $G_{as}$ .

The crystal structure of the *ras*-encoded p21 in its GTP-bound state has recently been determined by Pai and co-workers<sup>3</sup> at the Max Planck Institut in Heidelberg.

determined by Pai and colleagues, is that Thr 35 coordinates the magnesium ion in the binding site and hydrogen bonds to the  $\gamma$ -phosphate of GTP. This region of p21 is likely to undergo a conformational change when GTP is hydrolysed to GDP, and may thus allow effector molecules to distinguish between GTP- and GDP-bound forms of p21. GAP, for example, binds to p21 only in the GTP-bound form<sup>6</sup>. In  $G_{as}$  the equivalent residue is Ser 193. If this analogy is correct, mutations at  $G_{as}$  residues equivalent to those defining the *ras* effector-binding region (such as Pro 192, Asp 196) might interfere with adenylyl cyclase activation. Also, if the structures of p21 and  $G_{as}$  are similar in this region, then it is unlikely that Arg 201 could form part of the GTPase site of  $G_{as}$  as it would be a considerable distance away from the bound nucleotide. We would then predict that mutations at this site, or ADP-ribosylation by cholera toxin, would produce conformational changes in the

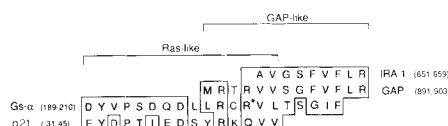


FIG. 1 Comparison of GTP-binding protein sequences using single-letter codes.

$G_{as}$  protein that would affect GTPase indirectly.

An alternative possibility is that Arg 201 is actually part of the GTPase active site of  $G_{as}$ . This would be consistent with the effects of mutagenesis or ADP-ribosylation at this position, if not with structural predictions based on p21. Pursuing this possibility, we reasoned that GAP might directly contribute to the GTPase active site of p21. We therefore searched GAP sequences for similarities with the  $G_{as}$  201 region. When such a sequence was identified (G. Martin, Cetus), it was found to be in a region of GAP which is similar in sequence to the protein encoded by the yeast gene *IRA1*. This gene down-regulates the function of *RAS2*, a *ras*-like gene in yeast, possibly by stimulating GTPase activity<sup>8</sup>. Indeed, human GAP can replace the *IRA1* protein in yeast (R. Ballester, personal communication). It is, therefore, possible that these conserved regions of GAP and *IRA1* are sites of interaction with *ras* and *RAS2* proteins, and that for GAP at least, this region includes amino acids that contribute directly to the GTPase site. Clearly this possibility can be tested by mutagenesis in this region of the gene encoding GAP. For example, alteration of GAP residues Arg 894, Val 895 or Phe 901 might prevent GAP-mediated p21 GTPase activity.

Apart from raising questions concerning the relationship between Gsp, *ras* and GAP and focusing attention on the 201 region of Gsp/ $G_{as}$ , the work of Bourne and colleagues has two other important implications. First, the consequences of constitutive cAMP production can now be investigated in the context of cellular transformation. For example, transcription of many genes (including *c-fos*) is controlled by the action of cAMP-dependent kinases. Production of some of these gene products may be essential to malignant transformation. Proteins that control progression through the cell cycle are themselves controlled by phosphorylation. Cyclic AMP may have direct or indirect effects on these events. It may therefore be possible to trace pathways involved in oncogenesis beyond the second messenger in these cells.

Second, the possibility that other G proteins may be activated by point mutations must be considered. Most cell types do not proliferate in response to cAMP, so the spectrum of tissues responsive to Gsp may be limited. However, the effects of

$G_i$ ,  $G_o$ ,  $G_z$  and others (reduced cAMP, increased phospholipases, altered ion flux and so on) may contribute to transformation in many other cell types. The oncogenic potential of these pathways has been revealed by reports that muscarinic receptors are mitogenic in neural cells (these receptors work through a  $G_i$  protein, see Hanley's News and Views article<sup>9</sup>), that ectopic expression of the serotonin-1c receptor (which is coupled to an unidentified G protein) is oncogenic<sup>10</sup>, and the identification of the *mas* oncogene as an activated receptor<sup>11</sup>. In each of these cases it is possible that activation of the G

CYCLASE ENZYMES

## Deciphering the mosaic

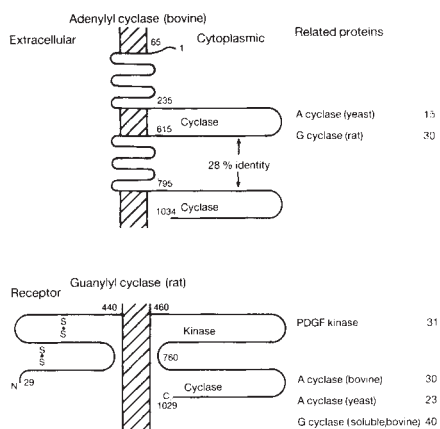
N. Michael Green

Hot on the heels of the first sequence of a guanylyl cyclase<sup>1</sup>, has come that of adenylyl cyclase<sup>2</sup>. Its protein sequence, deduced from the DNA sequence, reveals an unexpectedly large proportion of hydrophobic transmembrane segments clustered in two groups of six, each alternating with 300 residue cytoplasmic domains, which show 30 per cent mutual identity, and a similar degree of resemblance to the family of guanylyl cyclases.

Such a mosaic of loose correlations is typical of many receptor proteins, but although adenylyl cyclase is membrane bound, it is not thought to be a receptor and it does not respond directly to hormonal stimuli. Elucidation of the pathway of activation of adenylyl cyclase via a separate membrane-bound receptor and a soluble cytoplasmic G-protein complex (see Fig. 2b of McCormick's News and Views article, opposite) has followed from the initial observations of Rodbell *et al.*<sup>3,4</sup>. Progress in understanding the role of G

protein, rather than of the receptor, could lead to malignant transformation. We may therefore expect to see a new wave of oncogenes, many with restricted cell specificity, and some with known biochemical functions. The pathways that these proteins control are certain to interact with those controlled by more familiar oncogenes, and should help the painstaking process of identifying biochemical events that are critical to oncogenesis. □

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Comparison of the organization of domains in membrane-bound adenylyl and guanylyl cyclases. Percentage identities to domains of other proteins are indicated on the right. The guanylyl cyclase receptor domain has 33 per cent identity with the atrial natriuretic peptide receptor. PDGF, platelet-derived growth factor.

proteins was accelerated following appreciation of their involvement in the responses of rhodopsin to light. The new sequence brings with it the possibility of describing the coupling of receptors to adenylyl cyclase in similarly detailed molecular terms, especially if the cyclase can be expressed and studied by mutagenesis.

The history of guanylyl cyclases has followed a different but almost equally tortuous course<sup>5,6</sup>. Coupling of receptors to the cyclases is direct; G proteins are not involved. The recently cloned guanylyl cyclase from rat brain<sup>1</sup> has only a single transmembrane segment which links it to a receptor for the atrial natriuretic peptide (see figure). Between the catalytic domain of the cyclase and the membrane is a domain that resembles the oncogene-associated tyrosine kinases, though it has not yet been shown to function as such.

More detailed examination of the sequence of adenylyl cyclase shows that the pair of similar cytoplasmic domains resemble the catalytic domains of membrane-bound and soluble guanylyl cyclases more closely than they resemble the adenylyl cyclases of yeast and prokaryotes (see figure). The two clusters of transmembrane segments are similar to each other in overall content of polar residues (8–10 charges or amides) but there is no parallel between the locations of the charges nor between the lengths of loops between the segments.

The cyclase domains of all the kinases show significant similarity of sequence and a predicted secondary structure in which helices and strands tend to alternate, though not in any regular fashion. There is no indication of any sequences or secondary structure patterns characteristic of nucleotide-binding domains, such as those found in the protein-kinase domains. Krupinski *et al.*<sup>2</sup> draw attention to an adenylyl cyclase sequence, GAGESG, that is similar to the conserved first loop of

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a nucleotide domain, but as this segment is part of the long, glycine-rich, extracellular, amino-terminus, which has no strands or helices in the neighbourhood, it cannot have anything to do with nucleotide binding. It is a common error to assume that the sequence of a short segment is sufficient to define a potential nucleotide site. Sequence and/or secondary structure should match over at least 100 residues before these assignments can be made with any confidence<sup>7,8</sup>.

Neither of the cyclases has yet been expressed in a functional form. Nevertheless, their sequences enable one to ask much more precise questions about function and control, and to search for similar features in other proteins. A duplicated structure, similar to that of adenylyl cyclase, has been observed in the multi-drug resistance P-glycoprotein<sup>9</sup>, where the transmembrane domains may function as a transport channel. Krupinski *et al.* suggest<sup>2</sup> that the transmembrane domains of

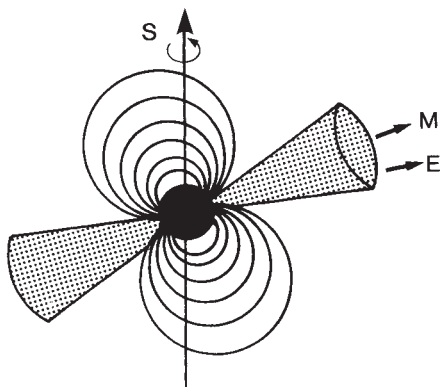
adenylyl cyclase might be required for export of cyclic AMP following its synthesis by the enzyme and performance as an intercellular signal, but direct evidence is lacking. Hydroxymethyl glutaryl CoA reductase is another enzyme that combines a catalytic domain with an extensive transmembrane domain, but no clear function has been found for the latter either. □

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## Rocket-propelled neutron stars

How can neutron stars, which we observe as pulsars, acquire velocities of 300 km s<sup>-1</sup> or more? These high velocities, or rather their transverse components as seen projected on to the sky, are measured either geometrically as proper motions or indirectly by observing the rate of fluctuation of their radio signals as the pulsars move through the ionized interstellar medium. The origin of these high speeds may be either at the birth of the neutron star or later, when they are acquired through a rocket acceleration process (see figure). P. Yu. Pskovsky and



The geometry of a pulsar, and in particular the direction of the spin axis in relation to the direction of the Earth E, can be deduced from the characteristics of the radio beam, which is centred on the magnetic pole M.

O. F. Dorofeev propose elsewhere in this issue (*Nature* 340, 701–702; 1989) a test for distinguishing these two and show that the evidence favours a rocket process.

The test is based on the reasonable assumption that the direction in which a rocket acts is associated with the rotation axis of the pulsar. Fortunately we do have some good information on the direction of the rotation axis. The detailed characteris-

tics of the radio pulses, and in particular their polarization and width, depend on the angle between the rotation axis and the line of sight which should determine the projection angle between the rocket and the plane of the sky. A reasonable body of data already exists for a correlation to be sought between the observed proper motion and the inclination of the rotation axis. If the acceleration theory is correct, the observed component of the velocity should be greater for large inclinations.

A positive result would be a surprise, as a related test involving direction of proper motion rather than amplitude has already failed to find any correlation (B. Anderson & A. G. Lyne *Nature* 303, 597–599; 1983). Pskovsky and Dorofeev do indeed surprise us by showing that there is a good correlation between velocity and the inclination of the axis. The two sets of data which are found to be correlated, although uncomfortably small, are obtained in entirely separate observations and could not be accidentally related in any indirect way. They are deductions from some difficult measurements and we must of course check the analysis on another set of pulsars.

If the correlation is proved to be good we have an answer to a question which has been with us since 1975, when E. Tademaru and E. R. Harrison (*Nature* 254, 676–677) first proposed the rocket mechanism. The remaining problem would be to explain how a single jet, or an asymmetrical pair of jets could be produced from a spinning neutron star, which we usually think of as a simple sphere with a dipolar magnetic moment.

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## Film fun

SOAP-FILMS are the most surprising and non-intuitive of liquid structures. What a pity, says Daedalus, that they are so easily burst by mechanical damage! He now proposes a way of making them really robust.

Long-chain polymers are often added to soap-film solutions to increase their viscosity and hinder gravity-thinning. And some long-chain polymers can be made as molecular closed loops. So, says Daedalus, consider a soap-film thickened with such a polymer, and punctured at a point. The momentary breach must widen catastrophically under the surface-tension of the film — unless a closed-loop molecule happens to surround the initial hole. Then the hole can expand only by breaking the loop. Daedalus calculates that just one molecular loop-molecule about a micrometre across could withstand the pull of surface-tension, and save the soap-film. It would merely have a tiny pin-hole in it.

Even better, the pin-hole would soon heal up! In its random Brownian wriggling, the loop-molecule would sooner or later close up or cross itself, spreading the film once more over its central vacancy.

So DREADCO's chemists are formulating special soap-film liquids containing molecular-loop compounds. These big rings are so hard to synthesize that the first trials are using plasmids borrowed from DREADCO's molecular biology department. Fortunately it only needs a few parts per million of loop-molecules in a film to ensure that any puncture will find itself enclosed by at least one of them. Unlike traditional frail soap-films, this new film will be totally resistant to mechanical assault.

Thus will be born DREADCO's 'hyper-foam', a resilient unbreakable air-filled cleansing foam with excellent energy-absorbing properties. Ordinary fire-fighting foam-blowers could rapidly generate it in vast amounts. People could safely leap into it from the upper stories of a burning building; aircraft landing in emergency and racing cars out of control could plough into it and be safely dragged to a stop. The military would love it as a sort of anchored smoke-screen, and the police could use it as a humane way of discouraging riots and street clashes.

Immersion in Hyperfoam could be a quite sensual experience. If made isotonic with human lung fluid, it could be breathed safely and even pleasantly; it would diffuse light, deaden sound, and retain heat. A full-submersion Hyperfoam bath, or even a whole Hyperfoam room, combining hygiene, warmth, and mild sensory deprivation, would be an appealing luxury. And Hyperfoam thinned below a light-wavelength to the invisible 'black-film' condition, would seem like a warm, sticky, viscous, caressing air. David Jones

# Dissipation in computation

SIR—All proposals for 'reversible' or dissipation-free computation<sup>1</sup> seem to me to have a common flaw: the discussion concentrates on what happens between the input and output of a gate (or a computer), but neglects what occurs at input and output.

Taking the billiard-ball model as an example, to initiate its operation requires the simultaneous start at  $t_0$  of several balls from their respective input channels (which determine the shooting direction). To this end the balls have to be inserted some time before  $t_0$  and held in place against thermal motion. This can be achieved by static friction between balls and tubes or by some kind of bolts placed in front of the balls and held in place by static friction so that they cannot give way prematurely by their own thermal motion. Equally, the driving springs have to be loaded before  $t_0$  and secured behind the balls by appropriate bolts. Even neglecting all the energy dissipated during these preparations, to initiate our computation at  $t_0$ , we have to withdraw all the bolts against static friction. This has to be done rapidly, or else  $t_0$  would be indeterminate, and so unavoidably we have to dissipate at least several  $kT$  of energy per input ball.

Equal amounts of energy have of necessity to be dissipated at every output. Any gadget that does not yield a recognizable output signal cannot be considered to be a valid computer model; a billiard ball that is elastically reflected at the 'output' without any loss of energy cannot yield any kind of output. Whether the balls, running against reflecting springs, are trapped at the reversal point by appropriate bolts—so that they can serve for read-out before they are let loose on their return trip—or whether they shift small signal-bolts at the output (which can be identical to the input-bolts of a subsequent gate), one cannot avoid the dissipation of several  $kT$  of energy per bit.

As for those models in which it is claimed that frictional losses could be reduced at will by simply reducing the speed of the balls correspondingly, when the forward-directed motion becomes slow compared with the average thermal motion, the passage of the balls from input to output becomes completely unpredictable; such a device is surely not a valid model for a computer.

Landauer<sup>1</sup> describes a Fredkin gate in which a ball pushes the two halves of a split control pipe apart. This ball must exert pressure on these control plates, and so will unavoidably experience static friction proportional to this pressure and independent of its velocity during its passage through the control pipe. The 'ideal viscous fluid', in which the whole affair is assumed to be immersed, will have no

effect; it will simply be driven away from between ball and plates. How can such a device be claimed to function without minimum energy dissipation?

Finally, I do not believe that 'unwinding the program' can reduce the energy dissipation that occurs when resetting the output register. No ball can arrive at the output that was not started somewhere as an input ball; therefore, the numbers of input and output balls being equal, it can make no difference whether we discard these balls from the output register or, after unwinding the program, from the input register. Landauer's proposal to avoid resetting losses altogether by (hopefully) dissipation-free copying and uncopied of the input is nothing but the simple transmission from the register into a store of (necessarily) infinite capacity. Now consider the energy required to produce all this hardware.

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LANDAUER REPLIES—Reversible computation tends to be counterintuitive at first exposure. Since its original description<sup>2</sup>, the concept has been elaborated by many with differing viewpoints, including R. P. Feynman<sup>3</sup>. Despite this widespread acceptance, criticism still appears in print, typically without citation of earlier similar debates. My rebuttal<sup>4</sup> of such a critique cites some of these previous debates. There are various possible realizations of reversible computations described in the literature: Biedermann focuses exclusively on the billiard-ball and Fredkin-gate versions. It is conceivable that one or other of the existing embodiments is flawed: demolishing one version is not an adequate rebuttal to reversible computation itself.

Biedermann focuses on the input and output operations in the billiard-ball model. As I have stressed in ref. 4, information transfer at input and output need not differ from that occurring within the computer. On the other hand, bit transfer at either end can easily be made more dissipative than that within the computer. Biedermann discusses such alternatives but does not tell us why he considers them to be optimal processes.

Most, but not all, reversible computers move back and forth in a diffusive fashion if watched over a short period, but with a predictable velocity over a long period, analogous to that of a brownian particle moving in a force field. Biedermann claims that "such a device is surely not a valid model for a computer". In my opinion, a system that carries out a computation is a computer. If the computer is going to dissipate much less energy than in current technology, it is likely to differ

substantially from conventional systems.

Biedermann's charges that I ignore static friction can be answered on four levels. First, we are following the time-honoured example in the discussion of thermodynamic cycles. Second, not all reversible computers depend on mechanical devices; Bennett<sup>5</sup> has proposed one based on genetic-code machinery, and Likharev's<sup>6,7</sup> version uses Josephson junctions. Third, in these discussions, we inquire about the limits imposed by physics, and not about what can be practically realized. We are not far from the capability to build devices with atomic precision and with perfectly periodic surfaces, preserved by operation at low temperatures. There will then be no static friction. Last, even in the absence of a lubricating film, and with irregular surfaces, I believe that there is a wide range of conditions that eliminate static friction, as long as the velocity is controlled (rather than the force) and is kept small relative to thermal velocities. For ordinary macroscopic devices such a velocity is uselessly small.

Biedermann points out that the number of information-bearing degrees of freedom is conserved during reversible computations. Both the number of 'balls' and the information in the output reflects that in the input. But in the input most of the information is in the form of a supply of balls in standardized states; the variable information is confined to the program. After a long computation the final state of a reversible computer has many more balls whose state depends on the computation that has occurred.

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## Space sickness on Earth

SIR—We report here the surprising after-effects of prolonged centrifuge runs in which we, the three scientist-astronauts on board the D-1 Spacelab mission (US Space Shuttle Challenger flight, 30 October–6 November 1985), have participated. We think we can simulate the space adaptation syndrome, better known as space sickness, on Earth. Such a method has, to our knowledge, not previously been reported, nor has a correlation been found between individual susceptibility to space sickness and Earth-bound motion sickness. For these reasons, it has been difficult to study the cause of space sickness or to develop preventive measures



involving selection, training and/or medication.

To be sick during the first days in space is quite normal. More than half of the astronauts are sick initially, but after two days almost all of them feel better<sup>1</sup>. Space sickness and adaptation are of both operational and general scientific interest. Space sickness can play a particularly negative role during short missions of a few days, and can also be hazardous during early extra-vehicular activities (in space suits).

Generally speaking, its cause is related to the absence of gravity. The study of space adaptation may therefore provide greater insight into our vestibular system, our posture, our visual reflexes and the interactions between them. Quite extensive research into these areas has already been conducted on the ground as well as in space<sup>2,3</sup>.

The method we report here was arrived at by chance. We were investigating the effects of prolonged acceleration on humans to 2 or 3 g, based on an experimental finding that the immune system is more active at above normal g and almost inactive at 0 g (ref. 4). As prolonged centrifuge exposures are not reported in the literature, we started carefully with extensive medical monitoring. To limit the stresses on the body, the supine position was chosen. It was this orientation relative to the acceleration vector that led to the surprising after-effects and the similarity to space sickness.

We all then participated in more systematic tests<sup>5</sup> in which each of us was exposed to 3-g acceleration for 1½ hours while lying on our backs. The very specific symptoms induced persisted for 5–6 hours. Even very slight head movements in pitch led to a strong sensation of movement, which was provocative. All three of us perceived the readaptation to the normal 1-g environment when coming from the 3-g environment in the centrifuge to be very similar to the adaptation to 0 g during the first hours of our Spacelab mission, although in space both pitch and yaw head motions are provocative. Our individual susceptibilities to the space-adaptation syndrome were also reproduced. It is important to note that the full range of symptoms, from 'not being sick' to 'acute sickness', was experienced.

We conclude that this method of prolonged 'loading' of a subject with an acceleration of 3 g opens up a new field of vestibular research, because of the

specific nature and the reproducibility of its effects. It is the first test in which the participants have observed strong similarities to their experiences during their initial hours in space. We found a strong sensitivity to prolonged acceleration in the fore-and-aft direction. It could therefore be relevant to note that all astronauts and cosmonauts have been launched in the supine position, which could have enhanced the space-adaptation problems

very early in the mission.

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## Amalgamated sequences

SIR—We have noticed an interesting similarity between the recently determined sequence of the poliovirus receptor<sup>1</sup> and the sequences of two other cell-surface

for alignment of the amino-acid sequence of the receptor with the two *Drosophila* sequences. We find strong reason to argue, therefore, that the poliovirus receptor is part of the same new class of immunoglobulin superfamily proteins that contain this arrangement of immunoglobulin-like domains.

The figure shows an alignment of V- and C-domains of the three proteins, with the immunoglobulin-fold consensus residues highlighted, obtained by the Align program and inspection. Alignment of the V-domains gave a similarity score of ~7 s.d. All three proteins contain an amino-terminal domain with properties characteristic of signal sequences<sup>2</sup>, but only the poliovirus receptor has putative trans-membrane and C-terminal cytoplasmic domains. Amalgam is expressed on *Drosophila* neuronal cells, and the poliovirus receptor is probably expressed on human motor neurons, as these are

destroyed on invasion of the central nervous system by poliovirus, resulting in paralysis. These observations suggest that the two proteins have related functions, probably in cell-surface recognition events.

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| ama1 | V | E | F | N | C | T | V | E | . | . | . | E | V | G | Q | L | S | . | V | S | W | A | K | R | P | S | E | S |   |
| pvr1 | V | T | L | P | C | Y | L | Q | V | P | N | H | E | V | T | H | V | S | Q | L | T | W | A | R | H | G | E | S | G |
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| ama1 | D | T | N | S | V | . | H | L | S | M | R | N | I | L | S | L | P | D | . | K | R | Y | N | . | V | T | V | T | E |
| pvr1 | . | . | S | M | A | V | F | H | Q | T | Q | G | P | S | Y | S | E | S | K | R | L | E | F | V | A | A | R | L |   |
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| pvr1 | G | A | . | . | E | L | R | N | A | S | L | R | H | F | G | L | R | V | E | D | E | G | N | Y | T | C | L | F | V |
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| ama2 | L | E | L | T | C | H | A | . | N | G | F | P | K | P | T | . | I | S | W | A | R | E | N | N | A | . | . | . | . |   |   |   |   |
| pvr2 | P | M | A | R | C | H | V | S | T | G | R | P | P | A | . | Q | I | T | W | H | S | D | L | G | . | . | . | H | M | P | N | T | S |

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| ama3 | A | E | L | E | C | S | V | . | Q | G | Y | P | A | P | T | . | V | V | W | H | K | N | G | V | P | . | Q | S | S | R | H | H | E |
| pvr3 | A | T | L | T | C | D | A | . | R | S | N | P | E | P | T | . | G | Y | N | W | S | T | T | H | G | P | L | . | . | P | P | F | A |
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| ama2 | V | M | P | A | G | G | H | L | L | A | E | P | T | L | R | I | R | T | . | V | S | H | R | M | D | R | G | G | Y | Y | C | I | A | Q | A |
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| ama3 | A | N | T | A | S | S | S | G | T | T | T | S | V | L | R | I | D | S | . | V | G | E | E | D | F | G | D | Y | Y | C | N | A | T | A |
| pvr3 | A | Q | . | . | . | . | G | A | Q | G | A | Q | L | L | I | R | P | . | V | D | K | P | I | N | T | T | L | I | C | N | A | T | A |   |
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Alignments of: top, the V-domains and, bottom, the C-domains of the amalgam homologue (dps) of *D. pseudoobscura*, amalgam (ama) of *D. melanogaster*, and the poliovirus receptor (pvr). Residue numbers for V-domains: dps1, 45–127; ama1, 42–121; pvr1, 43–117. Residue numbers for C-domains: dps2, 153–208; dps3, 243–307; ama2, 157–212; ama3, 247–311; pvr2, 162–225; pvr3, 262–316. Boxed residues are those which are present at least three times in the immunoglobulin superfamily alignment of ref. 3; con is a consensus sequence corresponding to residues present at least four times in the immunoglobulin superfamily alignment.

proteins, amalgam from *Drosophila melanogaster*<sup>2</sup> and its homologue from *D. pseudoobscura* (T.C.K., unpublished sequence).

Both *Drosophila* proteins have the characteristic fingerprints of the immunoglobulin fold<sup>3</sup>, containing a variable (V)-domain followed by two constant (C)-domains. The Align program<sup>4</sup> gave a similarity score of close to 9 s.d. (and amino-acid identities of 24 % and 22 %)

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# Ladies of inequality

Janet Browne

**Sexual Science: The Victorian Construction of Womanhood.** By Cynthia Eagle Russett. Harvard University Press: 1989. Pp. 245. \$25, £15.95.

DESPITE the racy title, *Sexual Science* is a serious book about a serious subject. Cynthia Eagle Russett is professor of history at Yale University, and author of a number of studies on the relations between science and society in nineteenth-century America. Previously, she has focused on the way that non-scientists absorbed the implications of darwinism and the idea of thermodynamic equilibrium.

In this new book, she turns to a more complicated intermingling of worlds and takes a long, hard look at nineteenth-century science itself in order to chart the diverse and often inconsistent views that were expressed in both Britain and North America about the physical and social state of women. Many readers will perhaps be grateful that no modern axes are publicly ground, although the author is fully conversant with the latest work in womens' studies. But this does not mean that her story is a happy one: on the contrary, it fully justifies her claim that scientific work on sex differences during the Victorian period was neither unbiased nor value-free.

Philosophers, scientists, social theorists and the ordinary people in the nineteenth-century streets were all united in their belief that women were significantly different from men. Eagle Russett describes how these simple beliefs about basic biology were turned into major scientific concepts. The first section of the book, primarily following the work of Stephen J. Gould on the mismeasurement of skulls, is concerned with the way obvious anatomical differences were interpreted. The anthropological system of measuring skulls was used by biologists to show that the size of the female brain case was smaller than that of males (despite considerable evidence to the contrary), a conclusion that was thought to confirm the mental inferiority of women. Similarly, the popular science of phrenology codified all sorts of differences in the mental faculties of men and women.

These structural observations were

supported by a wide range of physiological findings. Taking up one of the great nineteenth-century achievements in the study of physiology, biologists such as Patrick Geddes and J. Arthur Thomson defined men as 'katabolic' entities, metabolically active, and women as 'anabolic',

IMAGE  
UNAVAILABLE  
FOR COPYRIGHT  
REASONS

The 'New Woman' of the 1880s and 1890s — "upbraided for over-developing her mind to the detriment of her role as a mother . . .".

passive and constructive. On another intellectual plane, Darwin worked strenuously to answer the question of why women were not as hairy as men — presumably fingering his own patriarchal beard as he did so.

Evolutionists began to think that women were 'undeveloped' men, arrested on the way to some supposed better state. Doctors found that women were more 'nervous' than the opposite sex, prone to a whole range of mental diseases that did not afflict men (Eagle Russett misses the chance here to talk about hysteria — literally, a mental state caused by disorders of the womb and by definition found only in women).

*Sexual Science* is particularly good in explaining the idea of a division of labour that so clearly lies behind all these statements of difference. Human bodies were thought to mirror the body politic. Henry Maudsley and others in Britain and the United States insisted that the natural economy of the female body could not accommodate both brains and babies: the 'New Woman' of the 1880s and 1890s was upbraided for over-developing her mind to the detriment of her role as a mother (probably, as Edward Clark darkly intimated, women were graduating from college with undeveloped ovaries). Directives from the professional scientific

community bombarded educationalists, demanding that they should avoid over-specialization in girls' tuition for the sake of the future of the human race. All the fears that Eagle Russett rather grandiloquently calls the "cosmic nightmare" of the closing years of the century came to rest on the idea that women without children were betraying their natural biological role.

Yet Eagle Russett leaves the pursuit of interlocking turn-of-the-century themes such as degeneration and eugenics to others: her interests expressly lie in mapping the construction of a particular view of women rather than in its possible consequences. Within this particular view, it ought also to be said that Eagle Russett is class-specific. Her book is concerned only with middle-class women. There is no indication of whether scientists had any comparable theories about working-class women, or whether the mass of working people was beyond scientific concern. Equally, one wonders what effect Queen Victoria had on the theories of

men who thought women were not made to rule. Moreover, apart from a passing reference to the 'Hottentot Venus', a famous fairground exhibit in Napoleonic times, women of diverse racial origins are not discussed. It would have been illuminating to have some parallels, if any, drawn with the state of science and society in France, for example.

But this is a useful text, full of interesting ideas and pertinent examples. It will add considerably to our understanding of worlds that have gone before. □

Janet Browne, University Library, West Road, Cambridge CB3 9DR, UK, is an Associate Editor of *The Correspondence of Charles Darwin*, published by Cambridge University Press.



# Diversity and Division

Roy G. Burns

**Mitosis: Molecules and Mechanisms.** Edited by J.S. Hyams and B.R. Brinkley. Academic: 1989. Pp. 350. £45, \$89.50.

In the preface to this timely book, the editors comment that they have been told "that this ... will be the last book on mitosis ... before everything is understood". By contrast, Dick McIntosh notes that "Mitosis is a tough nut to crack. The people lucky enough to have found an interest in this subject will not soon be out of work." Ten chapters separate these comments and amply illustrate why they are not mutually exclusive.

The striking feature of this collection of reviews is the stress on summarizing the available evidence, rather than the work of the individual authors. For example, Kent McDonald draws attention to the diversity in structural organization, noting that the spindles of some 280 organisms have been examined. This message is reinforced in separate reviews of the centrosomes and the kinetochores, and is a reminder that any successful model must be compatible with the observed structural diversity. The concept of a single underlying mechanism, with specific variations in different organisms, is surely valid, a view which Fernando Cabral notes is supported by the genetic evidence.

Understandably, most of the discussion of this mechanism relates to anaphase, during which the chromosomes move towards the separating poles, with emphasis on the behaviour of specific classes of spindle microtubules. It is unfortunate, despite the clear efforts of the editors to cross-reference the separate contributions, that some topics, such as the polarity of the microtubules and microtubule capture by kinetochores, are duplicated in successive chapters.

The concentration on anaphase movement, both *in vivo* and using *in vitro* models, paradoxically shows how little is understood about the other stages of mitosis, such as the highly dynamic and erratic 'jiggling' of the chromosomes during pro-metaphase, or the mechanism for ensuring that the duplicated chromosomes segregate to opposite poles. The inclusion of Peter Hepler's contribution on the membranes present in spindles is a refreshing acknowledgement that microtubules may not be the only important structural component.

The book's subtitle, *Molecules and Mechanisms*, highlights how few spindle proteins have yet been characterized. For example, the centrosome of animal cells and its associated centrioles remain an

osmiophilic cloud, illuminated by immunofluorescence with a variety of adventitious antibodies, yet it regulates microtubule nucleation and possibly also modulates microtubule disassembly. Similarly, the co-localization of calmodulin with some spindle microtubules, together with the evidence for local and temporal fluctuations in the free-calcium concentration, point to a fascinating yet still elusive story, while the search for molecular motors and other microtubule-associated proteins has only just begun.

*Mitosis* will certainly be essential reading for Dick McIntosh's "lucky people", who may not want to be reminded of those half-forgotten observations which challenge a favoured model. It should also be

read by people looking for challenging problems, or who wish to be impressed by the sheer experimental inventiveness of successive generations of cell biologists.

The problem with studying mitosis is that it is a dynamic process, involving mechanisms which cannot be resolved by light microscopy. Yet electron microscopy requires the specimen to be fixed. This book is like a high-resolution electron micrograph of a mitotic spindle: although a lot of detail can be found in it, it is just a snapshot, *circa* 1989, of a dynamic process. □

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## In the beginning

Beverly Halstead

**Evolution and the Fossil Record.** Edited by Keith Allen and Derek Briggs. Belhaven, London: 1989. Pp. 265. £25.

ON the door of my study there is a quotation from Heather Couper, borrowed in turn from *Woodstock*: "We are stardust". The magic of that phrase illuminates the consequences of the elements' evolution in the nuclear furnaces of the stars. In like manner David Attenborough conveyed the excitement of evolution and the fossil record in his television programmes *Life on Earth* and *Vanished Worlds*. John Fowles encapsulated it all: "fossils are the poetry of evolution".

With such an inherently fascinating subject how can anyone go wrong, even in a textbook? With *Evolution and the Fossil Record*, Allen and Briggs show how. Here is a book aimed primarily at advanced students of the earth and life sciences, but also at scientists in other disciplines. It is not intended to convert anyone, although there are a few snippets to treasure — Devonobiomorpha, it seems, are a new group of centipedes.

The scene is set with "Evolution of the Universe, Stars and Planets", a remarkably dense chapter which to me was incomprehensible: the diagrams, the abbreviations, the equations were quite meaningless. The table of the cosmic abundance of the elements, in which the elements are listed only by their abbreviations, is plain irritating — we should not have to remember what they all are.

"Pre-metazoan Life" is more digestible, although again densely packed (and with no discussion of the origin of eukaryotes and the pioneering contributions of Lynn Margulis). For me the pick of the bunch is "The Origins and Radiation of the Early Metazoa", which includes a few pictures of peculiar organisms. Here too, though,

there are gaps in that the account of biomineralization is disappointingly brief. The fall from the Garden of Ediacara, the Cambrian explosion, is unconvincingly described as "a geologically abrupt event of great biotic significance".

Patterns of evolution and extinction in invertebrates, vascular plants and vertebrates, and catastrophes in the history of life, are all the subjects of well-turned review articles. These would have been appropriate pieces for *Palaeontology*, the journal, but not to my mind for the book's intended audience.

Two topic chapters are included, dealing respectively with the colonization of land (plants and invertebrates) and vertebrate flight (pterosaurs, birds and bats). These are popular final-examination questions, one imagines — or at least they will be in the future. The book ends with a minor piece entitled "Evolution, Creationism and Science Education", which has the same level of incongruity as the opening chapter.

Each contribution is a summary of the state of the art aimed at experts or students already deeply embroiled in the subject. Highly technical language is used throughout. The book is very tough going, and it is in this respect that I feel that Allen and Briggs have failed. If one has the tenacity to persevere, there is a great deal of interest to be found here. But the editors have demanded an unreasonable level of commitment from their readers. □

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### An older Babbage

In an editorial addition to Jack Meadows's review of *The Works of Charles Babbage* (*Nature* 340, 517; 1989), Babbage's date of birth was given as 1792, a date that is widely quoted in secondary sources. It is in fact only two years, not three, before bicentenary celebrations can begin of Babbage's birth on 26 December 1791.

# Seeing things

Stuart Sutherland

## Visual Processing: Computational, Psychophysical and Cognitive Research.

By Roger Watt. Lawrence Erlbaum: 1988. Pp.152. £14.95, \$26.95.

**Visual Cognition: Computational, Experimental and Neuropsychological Perspectives.** By Glyn W. Humphreys and Vicki Bruce. Lawrence Erlbaum: 1989. Pp.330. Hbk £19.95, \$37.95; pbk £9.95, \$16.95.

PSYCHOLOGISTS are better at discovering problems than at solving them. No sooner is a good clean theory put forward than someone rushes to the laboratory to obtain results that not only disprove it but also suggest a host of new problems demanding a totally different theory.

The late David Marr worked on a newly discovered problem, namely, how the brain obtains from the retinal image a labelled description of its luminance edges, a description that Marr called the 'primal sketch' and that must form the basis of all further visual processing. He suggested that two operations were needed. The image must be blurred, which can be achieved by taking the weighted sum of luminance over a region around a given receptor, and the second derivative of the blurred luminance distribution must be found; this function will show two departures from zero, one positive and one negative, on either side of any luminance step in the image. These operations can be achieved by filters of different sizes that respond to luminance changes over different extents. There remains the problem of how to locate the position of an edge from the output of the filters. Marr proposed that this is achieved by locating the points at which the second derivative passes through zero: as one moves across the image towards the edge of a dark figure, there will be a decrease in the second derivative followed by a rise as one moves inside the figure. Thus, the zero crossing gives the position of the edge.

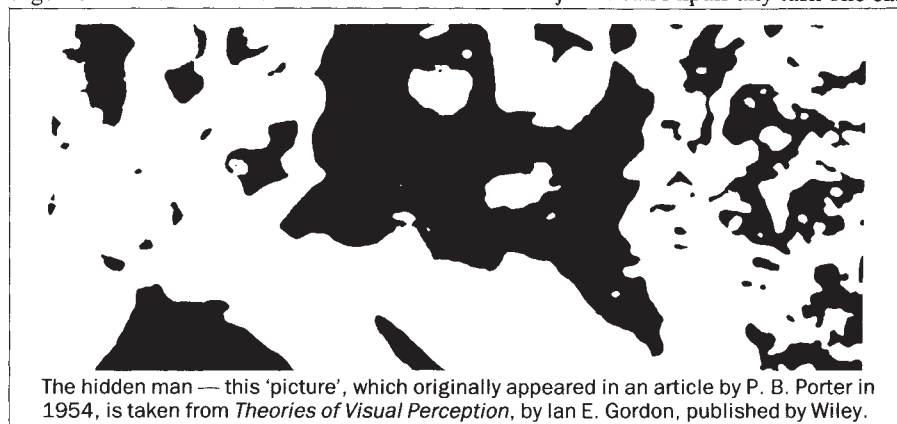
In *Visual Processing*, Roger Watt describes and defends a different and highly ingenious theory developed by himself and Professor Mike Morgan. He argues that where a luminance change extends over some distance, as for example in the penumbra of a shadow, marks on the surface or noise may produce zero crossings that will mask the zero crossing produced by the overall brightness change. His solution is to sum separately all positive outputs from the different sized filters and all negative outputs. In this way the overall change in brightness will be preserved as both for the positive and the negative outputs, the points at which the resultant curves touch zero are determined only by

the output from the largest filter. He argues that to detect even finer detail the largest filters are successively switched off.

Watt produces considerable evidence in favour of his theory, mainly from work on the contrast at which gratings of different widths can be seen and from the accuracy at which edges can be located. One wonders, however, just how far he has optimized the parameters of rival theories in making these comparisons, and also how far he has searched for data that disconfirm his theory.

He develops the theory to account for visual attention and the grouping of neighbouring items in the visual image. For example, on his theory a group of a dozen or so black dots randomly spaced but close together in the visual field will be imme-

diately detected as a group because the brightness difference between the region containing them and the background will be detected by the largest filter. The following *Gedanken* experiment suggests that at the very least this is not the whole story. Imagine a group of small black and white dots on a grey background. *Prima facie*, these will be just as readily seen to form a group as a region containing only black dots, but the broadest filter will not respond since there is now no overall brightness change. Marr's theory would account for this phenomenon, since he assembled groups from the individual members rather than proceeding in the reverse direction.



The hidden man — this 'picture', which originally appeared in an article by P. B. Porter in 1954, is taken from *Theories of Visual Perception*, by Ian E. Gordon, published by Wiley.

*Visual Processing* is imaginative and stimulating, though not easy reading, partly because of the intrinsic difficulty of the subject matter and partly because Watt often does not take sufficient pains to make himself clear. *Visual Cognition* is also difficult: it is very dense, has an uneven prose style and in places the undue number of typographical errors is distracting. In so far as the book has a theme, it would appear to be the recovery of meaning from the visual image. It does not deal in depth with many of the more traditional problems, such as colour vision, depth perception, the constancies or acuity, but concentrates on the primal sketch, object recognition, motion perception, imagery, visual attention and

think of and, of more importance, any combination of tasks. The use of such evidence for theory construction is clearly hazardous.

Despite the thoroughness of the reviews of each topic in *Visual Cognition*, one is left with qualms. The authors are interested mainly in the order in which different processes take place. It may well be that the existing evidence does not allow one to go beyond this, or even in many cases to reach it, but graphs containing sequences of boxes labelled "face identity units", "person identity nodes", "name generation" and so on, make one feel uneasy. It would after all be strange if one could put a name to a face without having recognized it. What one really wants to know is what goes on in each box. How are faces recognized and how are names generated?

Despite this reservation, the authors have done an excellent job in systematizing the existing evidence. The book will undoubtedly be useful as an advanced text for some years, indeed until such time as a host of new experiments has made it necessary to complicate even further their already complex exegesis. As they rightly remark, "Unfortunately the argument cannot stand there as the empirical evidence suggests a yet more complicated picture". It will continue to do so. □

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# The northern light

David W. Hughes

**James Joule: A Biography.** By Donald S.L. Cardwell. Manchester University Press: 1989. Pp.333. £35, \$59.95.

JAMES Prescott Joule, the Prescott being his mother's family name and the Joule, rhyming with cool, originating from the Derbyshire village of Youlgreave, was a Manchester/Salford man through and through. He was born on Christmas Eve 1818 into a famous brewing family and spent some of his early years working for the firm. Eventually his scientific interests predominated and it is said that the requirements of brewing technology and the accountancy needed to run a business helped to mould his scientific attitudes. A spinal weakness at birth turned him into a hunchback, and this shy and unassertive man was always sensitive about his public appearances.

Science in the time of Joule was changing from being the affair of the gentleman devotee to being the occupation of the full-time professional, ensconced in the university laboratory. Joule was in the first category. Almost all of his research was carried out in his laboratory at home and at his own expense.

According to my old science master, Joule's maxim was that "when you work you get hot". Reading Cardwell's biography one quickly realizes just why Joule is so often treated with such flippancy. His reticence often meant that his discoveries were attributed to more verbose and flamboyant researchers. Joule was a scientist's scientist. His main interest lay in exact measurement and his special genius showed itself at its best in the invention of methods for obtaining greater accuracy in quantitative experiments. He was systematic and hard-working — discreet about the origin of his ideas and cautious about his speculation.

Joule found that the heat generated by the flow of electricity was proportional to the electrical resistance multiplied by the square of the current. His experimental skills firmly established the law of conservation of energy. We take this law for granted now, but in Joule's time the complete conversion of heat into work or work into heat was no more conceivable than the conversion of gravity into hydrogen or hydrogen into gravity.

There was, too, his paddle-wheel experiment, which showed that any fluid could be heated merely by agitating it. One never forgets that because of this simple fact the water that has dropped the 49m over Niagara Falls is 0.11°C higher in

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James Prescott Joule — a scientist's scientist. (Mary Evans Picture Library.)

temperature than at the top. More precisely, one can state (in old units surely) that the work done in raising a weight of 1 lb through 772 ft will, if converted into heat, raise the temperature of 1 lb of water by one degree Fahrenheit. The ratio between the heat and the work was termed *J*, this symbol being introduced by Lord Kelvin (William Thomson) in honour of Joule, his friend.

Joule also noted that the adiabatic expansion of gases leads to cooling, on account of work being done against the intermolecular forces. Experimental investigation into this process, in conjunction with Lord Kelvin, then professor of natural philosophy at the University of Glasgow, led to the gradient of an isenthalpic curve being known as the Joule-Kelvin coefficient.

Joule died 100 years ago, and in this timely biography Cardwell has done full justice to his subject. Not only has he carefully placed Joule's work in the context of the scientific aspirations of his contemporaries, he has also delved deeply into a huge collection of primary material and thus produced a definitive picture of the man and his work. The end result is a book that is not only eminently readable but is also an excellent work of reference and a fascinating introduction to James Joule and to the state of British science in the mid 1800s. □

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# Tidal triggering of starbursts and nuclear activity in galaxies

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Gas distributed throughout a galaxy responds strongly to the tidal field of a companion during a merger. In some cases dynamical instability will drive a large fraction of the gas into the inner regions of the galaxy. A strong burst of star formation will follow and subsequent evolution may lead to the formation of a black hole. Continued accretion of gas by the black hole may provide sufficient power to explain quasars and nuclear activity in otherwise normal galaxies.

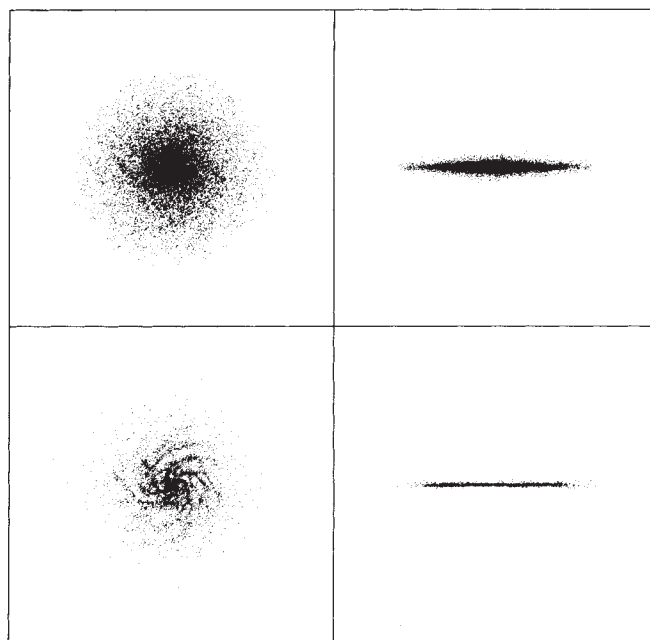
A FEW per cent of spiral galaxies display unusual behaviour in their nuclei, as evidenced by their intense infrared output and broad emission-line spectra. Infrared luminosities  $>10^{12} L_{\odot}$  have been detected (by the Infrared Astronomical Satellite, in the brightest galaxies<sup>1-3</sup>) to originate from regions  $<500$  parsecs in size. Similar integrated luminosities are commonly observed in the active nuclei of Seyfert galaxies, although, as suggested by time-variability arguments, the source of the radiation is even more compact—confined to  $\ll 1$  pc. It is generally believed that bursts of star formation are responsible for the infrared radiation, whereas active galactic nuclei (AGN) result from accretion of matter onto central black holes<sup>4</sup>. The mass required to account for the observed luminosity is not trivial, even by galactic standards. Gas concentrations in the range  $10^8$ – $10^{10.5} M_{\odot}$  have been inferred in a number of starburst galaxies<sup>5-10</sup>. Total fuel masses  $M_{\text{fuel}} \geq 10^8$ – $10^9 M_{\odot}$  are almost certainly required to power AGN over their lifetimes, given the

relative inefficiency of mass conversion into energy. In extreme cases,  $M_{\text{fuel}} \sim 10^{10} M_{\odot}$ , especially if the black hole forms from the accreted material. It is unlikely that the fuel could be primordial because the inferred depletion timescales are typically much less than a Hubble time<sup>11</sup>; hence, an external supply seems necessary. As emphasized especially by Gunn<sup>12</sup>, the mechanism by which this material is transported into the centres of galaxies and loses its angular momentum is poorly understood.

Here I adopt the point of view that the fuel is supplied directly as gas and that its source is the disk of the host galaxy. Simple arguments indicate that viscous processes alone are not sufficient to drive large quantities of gas into the nucleus<sup>13</sup>. Most models have instead relied on non-axisymmetric perturbations to the potential<sup>12,14,15</sup>, including bars and oval distortions<sup>13,14</sup>, tidal fields<sup>16-19</sup> and spiral density waves<sup>20</sup>.

Many observations suggest that star-formation rates are enhanced during galactic encounters<sup>21-24</sup>. The situation for AGN is unclear: recent studies<sup>11,25,26</sup> contradict earlier claims<sup>27-29</sup> that Seyfert galaxies are frequently associated with large nearby companions. Here, I report on the initial stages of an investigation of a related, but logically distinct mechanism for triggering activity in galactic nuclei: merging of galaxies. Observations indicate that up to  $\sim 20\%$  of Seyfert galaxies have amorphous morphologies<sup>26</sup>, perhaps indicative of recent accretion events, and seem to possess a clear excess of faint companions<sup>25</sup>. Motivated by these considerations I consider mergers in which the companion is much less massive than the host galaxy. My results demonstrate that mergers can lead to the accumulation of large central masses of gas in the nucleus of the primary galaxy, even if the companion is gas free. The final catastrophic inflow results from instability induced in the gas by the decaying satellite. In

FIG. 1 Distribution of stars (top, face-on and edge-on) and gas (bottom, face-on and edge-on) in an isolated disk galaxy after five half-mass rotation periods. Here, the number of particles representing stars is  $N_{\text{stars}} = 24,576$  and the number representing gas is  $N_{\text{gas}} = 8,192$ . The system timestep and gravitational softening parameter are  $\Delta t = \tau_{1/2}/100$  and  $\epsilon = 0.3$  kpc, where  $\tau_{1/2}$  is the rotation period at the half-mass radius. Timesteps for individual particles can be much smaller, depending on the local properties of the system. The gravitational-force calculation is performed using the hierarchical tree method with critical opening angle  $\theta = 0.8$  and including terms up to quadrupole order. Each panel measures 20 radial scale lengths per edge (70 kpc).





this phase, masses  $>10^9 M_\odot$  can be deposited into the inner few-hundred parsecs on timescales  $\leq 10^8$  yr, naturally explaining the apparent discrepancy between burst times  $\tau_{\text{burst}} \leq 10^8$  yr and galactic dynamical times  $\tau_{\text{dyn}} \sim 10^9$  yr (refs 30–32).

## Method

My simulations were performed using a hybrid  $N$ -body/hydrodynamics code<sup>33</sup>. The self-gravity of the system is calculated using a hierarchical tree algorithm<sup>34</sup> that was developed and optimized for vectorizing supercomputers<sup>35–37</sup>. Because the tree method is gridless and has a cost that scales with the particle number as  $N \log N$ , a relatively large  $N$  can be used without grid-based limitations on the spatial resolution or global geometry. Hydrodynamic properties are calculated using a variation of the Monte-Carlo technique known as smoothed-particle hydrodynamics (SPH)<sup>38–40</sup>. SPH is fully lagrangian and represents fluid elements computationally as particles. Each particle carries along with it local thermodynamic and hydrodynamic information, including the mass density  $\rho$ , the velocity field  $\mathbf{v}$  and the thermal energy  $u$ . Interpolation using a smoothing kernel permits these quantities and their derivatives to be estimated at any point in space. In my implementation, smoothing is performed with a cubic-spline kernel and the smoothing length is spatially variable<sup>33</sup>. Each particle has its own timestep, chosen to satisfy the Courant condition locally, so that large density contrasts can be resolved dynamically<sup>33</sup>. The pressure is given by the ideal-gas law  $p = \rho u (\gamma - 1)$  where  $\gamma$  is the ratio of specific heats. The lagrangian hydrodynamic conservation laws serve as equations of motion for the particles, and include artificial viscosity terms to capture shocks and other non-adiabatic sources and sinks of energy. For the simulations here  $\gamma = 5/3$  and the mean relative molecular mass is taken to be  $\mu = 0.72$ , as for a singly ionized gas of solar composition. Radiative effects are included by using a standard cooling curve<sup>41</sup>, and the source terms mimic the effect of heating by cosmic rays and the formation of molecules on grains<sup>42</sup>. In addition, cooling is suppressed below a temperature  $T_c$  to inhibit local Jeans instability (manuscript in preparation). Here, the cooling cut-off is always  $T_c = 10^4$  K. Further calculations show that the conclusions below are not limited to this choice (manuscript in preparation). Effects associated with star formation and supernova heating are ignored.

The mass distribution for the host disk galaxies is taken from the Bahcall–Soneira model for our own galaxy<sup>43</sup>, that is,

$\rho(R, z) = \rho_0 \exp(-R/h) \text{sech}^2(z/z_0)$ . The gas and stars are initially coextensive in radius with horizontal scale length  $h = 3.5$  kpc and the disks are truncated at radius  $R = 21$  kpc. The vertical scale length  $z_0$  of the stars is constant across the disks and is 700 pc. Initially, the gas is distributed with  $z_0 = 100$  pc and uniform temperature  $T = 10^4$  K, although it adjusts rapidly and settles into a quasi-static distribution in a few cooling times. The total mass of each host galaxy is  $5.5 \times 10^{10} M_\odot$  and the ratio of star-to-gas mass is always 10:1. The stars are given sufficient velocity dispersion to guarantee axisymmetric stability<sup>44</sup> with the Toomre  $Q$  parameter = 1.3 at the solar radius  $R_\odot = 8$  kpc.  $Q$  is defined<sup>45</sup> as  $\sigma_R \kappa / 3.36 G \Sigma$ , where  $\sigma_R$  is the radial-velocity dispersion,  $\kappa$  is the epicyclic frequency and  $\Sigma$  is the surface density. The disks are embedded in non-singular isothermal haloes of scale length  $a_{\text{halo}} = 3.5$  kpc and asymptotic rotation velocity  $v_\infty = 220 \text{ km s}^{-1}$ . For these parameters, the rotation curve is flat beyond  $R \approx 2h$  and the halo-to-disk mass ratio for  $R \leq 21$  kpc is 3.25. The halo is rigid, but moves in response to the satellite perturbation to conserve total momentum. The companions are modelled as spherical Jaffe galaxies<sup>46</sup> having  $\rho(r) = M_c a / [4\pi r^2(r+a)^2]$  with scale length  $a = 1$  kpc, and the disk-to-companion mass ratio is always 10:1.

## Isolated disks

An example of the distribution of stars and gas in an isolated disk, after five half-mass rotation periods, is shown in Fig. 1. A spiral structure develops in both components, but is most noticeable in the gas, owing to dissipation. Because the gas remains dynamically cold, it acts to destabilize the disk and to amplify spiral perturbations. The number of arms is consistent with the interpretation that they arise from swing-amplified noise. A simple estimate in the epicyclic approximation predicts that the fastest growing mode has an azimuthal wavenumber  $m = 1/f$ , where  $f$  is the fraction of mass in the disk relative to the halo<sup>47</sup>. For the system in Fig. 1  $m \approx 4$  and a Fourier decomposition shows that this mode indeed has the largest amplitude (manuscript in preparation). Further simulations indicate that the Fourier spectrum is somewhat sensitive to the cooling cut-off parameter  $T_c$  and is broadened if the gas consists of several phases. If  $T_c = 10^4$  K, as in Fig. 1, the gas is nearly isothermal and its temperature is mostly in the range  $10^4 \text{ K} \leq T \leq 2 \times 10^4 \text{ K}$ .

Averaged over the disk, the gas radiates at a relatively steady rate and the energy loss from the system amounts to  $\sim 2\%$  of the total gas energy per rotation period. Vertically, the gas

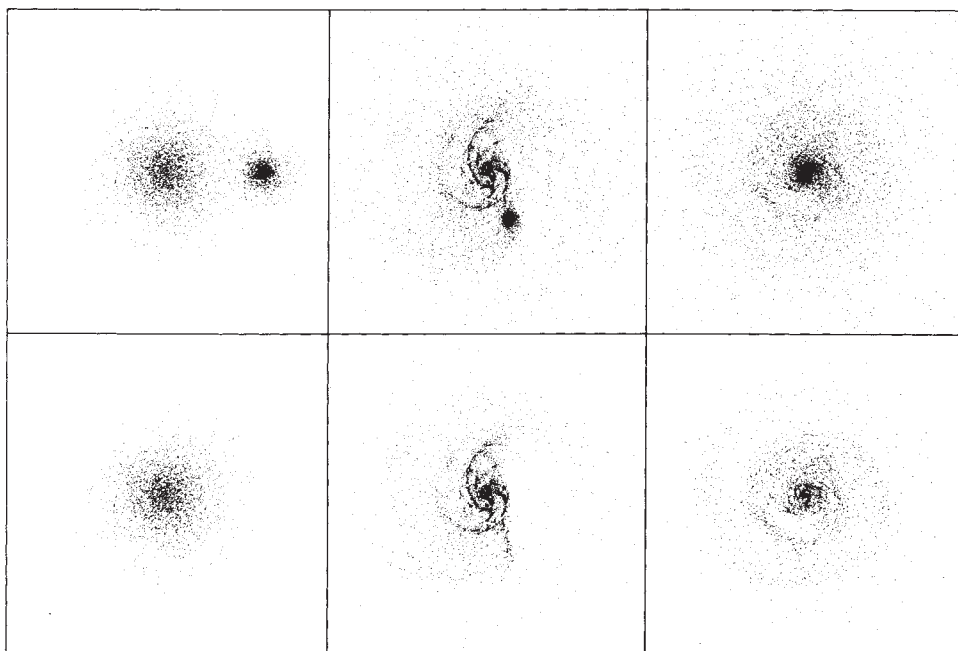


FIG. 2 Response of disk gas to decaying satellite. Top three frames show disk gas and satellite particles initially, at an intermediate time and at the final state  $2 \times 10^9$  yr from the beginning of the merger. Bottom three frames show the same view of only the gas. Here,  $N_{\text{gas}} = 4,096$  and the number of particles in the satellite is  $N_{\text{sat}} = 4,096$ . The stellar disk, not shown, consists of  $N_{\text{stars}} = 12,288$  particles. The timestep and parameters associated with the gravitational-force calculation are as in Fig. 1 and  $\varepsilon = 0.38$  kpc. Each panel measures 20 radial scale lengths per edge (70 kpc).

TABLE 1 Angular momentum of individual components

| Component   | $L_{1,500}$             | $L_{2,000}$             | $\Delta L$               |
|-------------|-------------------------|-------------------------|--------------------------|
| Central gas | $1.6843 \times 10^{-3}$ | $9.6506 \times 10^{-5}$ | $-1.5878 \times 10^{-3}$ |
| All gas     | $2.9485 \times 10^{-2}$ | $2.6822 \times 10^{-2}$ | $-2.663 \times 10^{-3}$  |
| Disk stars  | 0.34500                 | 0.35647                 | $1.147 \times 10^{-2}$   |
| Satellite   | $5.3539 \times 10^{-2}$ | $4.4708 \times 10^{-2}$ | $-8.831 \times 10^{-3}$  |
| Sum         | 0.42803                 | 0.42800                 | $-3.0 \times 10^{-5}$    |

Angular momentum of individual components in merger shown in Figs 2 and 3. Central gas refers to total angular momentum of gas particles ending up in concentration at centre of disk. Remaining entries are total angular momentum of all gas, disk stars, satellite particles and the sum of these three components. Entries for each component are angular momenta at  $t=1,500$  and  $t=2,000$ ,  $L_{1,500}$  and  $L_{2,000}$  respectively, and the difference  $L_{2,000} - L_{1,500}$ . Values are given in units where the gravitational constant  $G=1$ , time is measured in  $10^6$  yr and length is measured in kpc.

remains thinner than the stars with thickness determined by the gravitational softening parameter and the temperature of the gas.

Many of these findings are in good agreement with results obtained using a ‘discrete-cloud’ representation of the gas<sup>48</sup>. This agreement suggests that the global distribution of gas in disks is only weakly sensitive to its detailed phase structure.

### A merger

Here, the principal aim is to examine the response of disks, such as the one in Fig. 1, to mergers. An example is given in Fig. 2, which shows face-on views of the disk gas and a decaying satellite companion at various times during a merger. The satellite was initially on a prograde circular orbit at the edge of the disk, inclined by  $30^\circ$  with respect to the disk plane.

The orbit of the satellite gradually decays owing to dynamical friction. The disk responds strongly to the perturbation and shows varying structure as different internal modes become important. At intermediate times, the response is dominated by a two-armed pattern. The reaction of the disk stars to the tidal forcing is similar to that of the gas shown in Fig. 2, but somewhat less well defined (manuscript in preparation). The spiral structure in the gas is finer than in the stars because the gas can dissipate relative kinetic energy when it is compressed.

Following the merger, the disk has an overall amorphous appearance with little spiral structure evident in either the gas or stars. As first noted by Toomre<sup>47</sup>, and later demonstrated numerically<sup>48</sup>, this loss of spiral structure is simply due to dynamical heating of the disk stars.

Of greater interest is the accumulation of a dense central concentration of gas in the disk as a consequence of the merger, seen in the bottom panels of Fig. 2. In fact,  $\sim 35\%$  of all the gas distributed throughout the disk initially ends up in a region with a linear extent less than 400 pc.

### Radial gas inflow

The nature of the effect leading to the central accumulation of gas in Fig. 2 is most easily discerned by examining the inner regions of the disk during the final stages of the decay of the satellite, in Fig. 4. In the following discussion, time is measured in units of  $10^6$  yr.

As the satellite approaches the centre, its tidal field compresses both the gas and stars. The stars stream freely past each other, but the gas shocks and dissipates, forming a thin rod-like structure between  $t = 1,500$  and  $t = 1,530$ . Shortly thereafter, the orbit of the satellite moves it in a direction parallel to this linear structure and the tidal field now compresses the gas along the rod, between  $t = 1,540$  and  $t = 1,550$ . Eventually, the self-gravity of the gas comes into play and some of it forms a bound concentration between  $t = 1,550$  and  $t = 1,570$ . The continued influence of the tidal field of the satellite and the self-gravity of the gas result in a larger build-up as the bound concentration

accretes more gas. The gas blob begins to lose angular momentum to the disk stars through dynamical friction and it spirals towards the decaying satellite between  $t = 1,590$  and  $t = 1,630$ . By now, the gas concentration is so massive and dense that its tidal field dominates that of the satellite. In fact, the satellite is tidally disrupted by the gas between  $t = 1,630$  and  $t = 1,670$ .

The importance of the gas self-gravity is illustrated in Fig. 4, where the growth of mass in the central concentration  $M_g$  and the ratio  $W_{sg}/W_{vir}$  are shown. Here,  $W_{sg}$  is the gravitational potential energy of the gas blob in isolation and  $W_{vir}$  is the virial potential energy

$$W_{vir} = -2T - 3(\gamma - 1)U - W_{soft}, \quad (1)$$

where  $T$  and  $U$  are the kinetic and thermal energies of this gas, respectively, and  $W_{soft}$  is a correction term that accounts for softening (manuscript in preparation). The quantities  $M$ ,  $W$ ,  $T$ , and  $U$  are calculated from all the gas particles lying within 0.4 kpc of the single particle having the highest density at each time frame. Figure 4 also shows the ratio of the mass in disk stars to gas mass lying within this volume.

Nearly all the gas in the concentration accumulates between  $t = 1,500$  and  $t = 1,600$ . Continued accretion thereafter increases the bound mass by roughly 10% to  $\sim 1.7 \times 10^9 M_\odot$  at  $t = 2,000$ . It is also clear that the self-gravity of the gas is completely negligible until  $t \approx 1,500$  and does not become dominant until  $t \approx 1,550$ – $1,570$ . In other words, the onset of the instability results from hydrodynamical effects and is initially driven by the dissipative nature of the gas. Note that the ratio  $W_{sg}/W_{vir}$  does

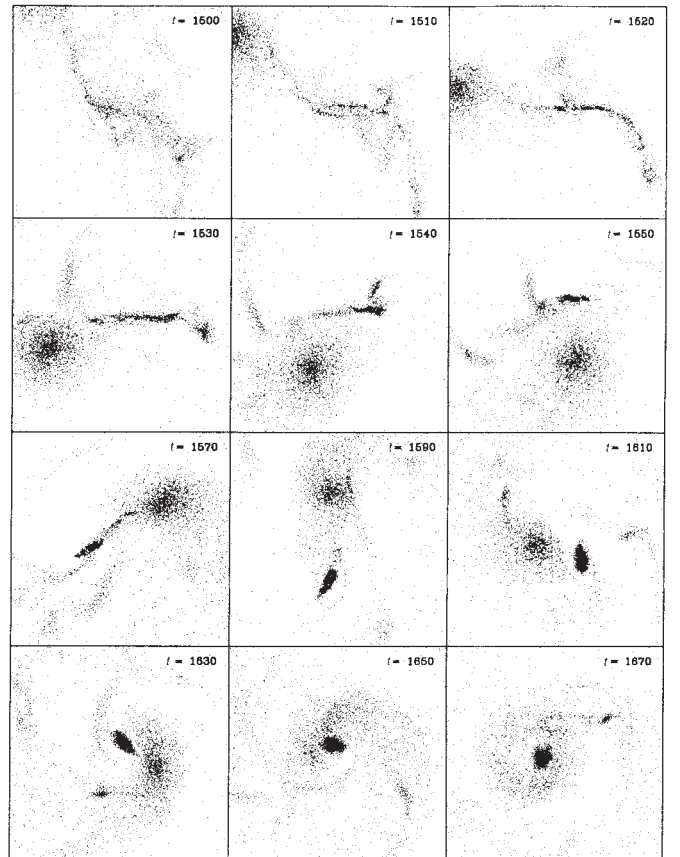


FIG. 3 Central regions of disk in Fig. 2 during final stages of merger. The disk gas and satellite particles are shown. Time, indicated in upper right-hand corner of each frame, is in units of  $10^6$  yr. First six panels are separated by  $10^7$  yr and subsequent ones are separated by  $2 \times 10^7$  yr. Each panel measure 2 radial scale lengths per edge (7 kpc). Satellite is located just above upper left-hand corner of first frame at  $t=1,500$ .



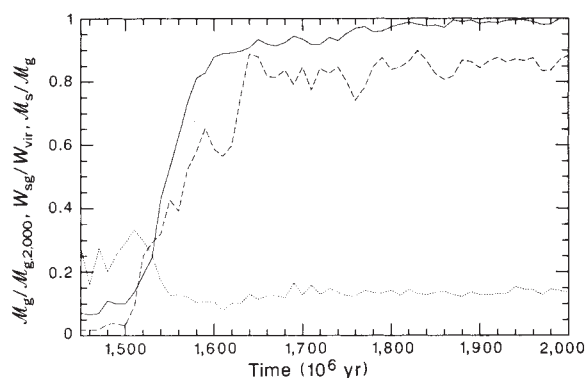


FIG. 4 Growth of mass in central gas blob  $M_g$  normalized to its value at  $t=2,000$  (solid line) as a function of time measured in units of  $10^6$  yr. Ratio of self-gravitating potential energy to virial potential energy for this gas  $W_{sg}/W_{vir}$  is indicated (dashed line). Importance of background potential is crudely given by ratio of mass in disk stars to the mass within central gas blob  $M_s$  to  $M_g$  (dotted line).

not approach unity exactly, as for a fully self-gravitating system, because of the background potential from the disk stars. The stars are also responsible for the rather large fluctuations in  $W_{sg}/W_{vir}$ , as shown by the ratio  $M_s/M_g$  in Fig. 4.

Perhaps most significant is the fate of the angular momentum originally carried by the gas ending up at the centre of the disk. Figure 5 shows the time history of the total angular momentum of just the gas particles lying within 0.4 kpc of the density centre at  $t=2,000$ . Also plotted are the total angular momentum of the gas, disk stars and satellite particles.

The net change in angular momentum from  $t=1,500$  to  $t=2,000$  for each of the components in Fig. 5 is given in Table 1. During this period, the angular momentum of the central gas decreases by a factor of roughly 17.5. The gas as a whole and the satellite particles also suffer a loss of angular momentum, with the only component gaining being the disk stars. Note that the sum of the angular momentum of the gas, disk stars and satellite is nearly constant from  $t=1,500$  to  $t=2,000$ .

The central gas loses angular momentum more or less monotonically, with some irregularity only between  $t=1,580$  and  $t=1,620$ . As the angular momentum of the satellite experiences a fluctuation of comparable magnitude but of opposite sign then, it seems likely that this behaviour results from tidal torques between the gas and satellite. Note from Fig. 3 that it is precisely during this period that the gas and satellite spiral towards one another.

Figure 5 strongly suggests that the primary mechanism by which the central gas sheds its angular momentum is gravitational coupling to the disk stars and satellite. Before the gas becomes self-gravitating, tidal torques are most important, but later, as the gas blob sinks to the centre, it also loses angular momentum by dynamical friction against the disk stars. To check that viscous transport is indeed much less significant, I binned the gas particles radially at  $t=2,000$  and calculated the time evolution of the angular momentum of the gas in each zone between  $t=1,500$  and  $t=2,000$ . Only the outlying gas beyond  $R > 10$  kpc acquired angular momentum—a small gain of  $\Delta L = 3.3 \times 10^{-4}$ , probably by gravitational coupling to the inner disk and satellite. The conclusion that angular momentum is redistributed gravitationally from the gas to the disk stars and satellite is most welcome and reassuring because the gas is only a small fraction of the total mass.

## Conclusions

The results of the preceding section demonstrate one mechanism by which tidal effects can lead to the deposition of gas at the

centres of disk galaxies. In the particular example presented here, a mass of gas  $\approx 2 \times 10^9 M_\odot$  is driven into a central region having an extent of roughly 400 pc on a timescale  $\leq 10^8$  yr, which is in good agreement with observed properties of starburst galaxies. Evolution beyond that shown in Fig. 3 is uncertain because star formation and supernova heating have been ignored. Given the high densities, a strong burst of star formation is inevitable. Once the gas is self-gravitating, fragmentation and instability should lead to further radial inflow, perhaps yielding an active nucleus<sup>4</sup>. As the disk is distorted, but not destroyed by the merger such events may explain some Seyfert galaxies. The most promising candidates are the amorphous Seyfert galaxies<sup>26</sup>, because of the generally featureless nature of the disk following mergers such as that in Figs 2 and 3.

The suggestion that small companions are responsible for triggering nuclear activity in some galaxies is not new and has been discussed previously<sup>12,49,50</sup>. The models presented here are, however, the first demonstration that mergers with satellites can deliver sufficiently large quantities of fuel to the central regions of disks to at least plausibly explain some occurrences of activity in galactic nuclei.

Further simulations demonstrate that these results are insensitive to the particle number and associated numerical diffusion, as well as to the form of the artificial viscosity. The latter conclusion is consistent with the inference that the angular momentum transport is driven mainly by gravitational rather than viscous processes. The results are clearly sensitive to the initial density profile of the gas in the disk and the structure of the companion. Because most of the gas ending up in the central concentration is initially within an inner region having a diameter of roughly two radial scale lengths, little activity would be triggered if the disk were gas-poor there. The satellite must be sufficiently dense to reach the centre of the disk, at least partially intact, before it is tidally destroyed. The calculation in Figs 2 and 3 used a satellite with a mean half-mass density twice that of the disk.

These constraints imply that merging alone probably cannot account for all occurrences of nuclear activity in galaxies. For example, the particular mechanism discussed here could not initiate activity in those early-type disks lacking gas in their central regions<sup>51,52</sup>. Also, it has not been demonstrated that the rate of merging with sufficiently dense companions is large

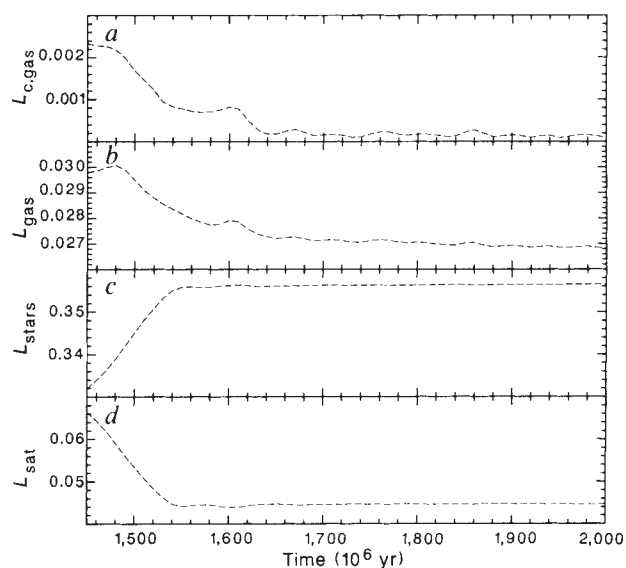


FIG. 5 Evolution of angular momenta of *a*, central gas *b*, all gas *c*, disk stars and *d*, satellite particles. Time is measured in units of  $10^6$  yr and angular momenta are expressed in units where  $G=1$ , length is in kpc and time is in  $10^6$  yr.

enough to explain the observed frequency of activity. Simple calculations indicate that typical disk galaxies have accreted up to  $\sim 10\%$  of their mass in the form of less massive satellites<sup>53</sup>. However, a reliable estimate of the distribution of satellite masses and densities will be possible only in the context of a complete theory for the formation of large-scale structure and galaxies.

The remaining uncertainties include the influences of the phase structure of the gas and star formation. The former is probably not critical provided that the gas can dissipate as it is tidally compressed. The typical separation between fluid elements in the gas rod that develops between  $t = 1,500$  and  $t = 1,530$  in Fig. 3 is  $\sim 100$  pc. The gas would still be expected to dissipate strongly at this point, even if it consisted entirely of discrete clouds, provided that the cloud radii are not much less than about 50 pc. Given that giant molecular clouds have sizes of this order, it appears that the detailed small-scale structure of the gas is unimportant. Star formation will undoubtedly modify the evolution and its neglect is probably the most serious weakness of the models presented here. Nevertheless, once the gas is drawn out into a thin rod-like structure it seems likely that a large fraction of it will become self-gravitating and fuel central activity. This could be prevented only if the gas were converted to stars at an exceptionally high efficiency before this stage, leading to a more extended starburst than suggested by Fig. 3.

The relative importance of mergers, as opposed to nearby transient encounters, for nuclear activity remains unanswered. As noted previously, current observations indicate that Seyfert activity is not correlated with galactic interactions<sup>11,25,26</sup>. Theoretical evidence on this question is ambiguous. Noguchi has

studied encounters of disks consisting of both stars and gas with other equal-mass galaxies<sup>19</sup>. He finds that pure parabolic collisions can induce bar instability in the stars, removing angular momentum from the gas and driving it into the inner regions of the disks. As noted by Barnes<sup>54</sup>, however, fully self-consistent encounters of equal-mass galaxies on initially parabolic orbits typically lead to merging within a few dynamical times unless the pericentre distance  $r_p$  is much greater than the extent of the haloes  $r_h$ . This condition is probably satisfied in Noguchi's calculations because he abruptly truncated his haloes at  $r_h = 20$  kpc and chose  $r_p = 40$  kpc. For more extended haloes, encounters with  $r_p = 40$  kpc will be highly inelastic and should lead to rapid merging. Noguchi was unable to examine such effects with his numerical approach. It appears that it will not be possible to establish a firm link between transient encounters and Seyfert activity without appealing to self-consistent models.

The scenario presented here predicts that the origin and evolution of activity in at least some galaxies is controlled by events on galactic scales. Such an interpretation is in accord with the suggestion that starbursting and nuclear activity represent different phases of the same process<sup>30-32</sup>. Furthermore, the frequency and intensity of activity should increase with the rate of merging and the fraction of luminous mass in the form of gas. This indeed appears to be true of observed active galaxies, which are more prevalent and luminous at high redshifts. Indeed, activity may be triggered during galaxy formation, if at least some of the mass accumulates in the form of discrete subunits. Ultimately, mechanisms like that described here may explain a much broader class of phenomena than aberrant behaviour in galaxies observed today, including quasars and extragalactic radio sources.  $\square$

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# GTPase inhibiting mutations activate the $\alpha$ chain of $G_s$ and stimulate adenylyl cyclase in human pituitary tumours

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A subset of growth hormone-secreting human pituitary tumours carries somatic mutations that inhibit GTPase activity of a G protein  $\alpha$  chain,  $\alpha_s$ . The resulting activation of adenylyl cyclase bypasses the cells' normal requirement for trophic hormone. Amino acids substituted in the putative *gsp* oncogene identify a domain of G protein  $\alpha$ -chains required for intrinsic ability to hydrolyse GTP. This domain may serve as a built-in counterpart of the separate GTPase-activating proteins required for GTP hydrolysis by small GTP-binding proteins such as p21<sup>ras</sup>.

MANY oncogenic mutations promote tumour growth by inducing autonomous activity of proteins that normally transmit proliferative signals initiated by extracellular factors. The role of cyclic AMP as an intracellular second messenger for several trophic hormones and its ability to stimulate growth of certain cultured cells<sup>1,2</sup> predict that oncogenic mutations should be found in genes for proteins that control its synthesis. Here we report identification of mutations that cause autonomous cAMP synthesis in four growth hormone (GH)-secreting human pituitary tumours. The mutations cause constitutive activation of  $\alpha_s$ , the GTP-binding subunit of the stimulatory regulator of adenylyl cyclase,  $G_s$  (refs 3–6), by inhibiting its GTPase. These mutations fulfill the specific prediction<sup>7</sup> that a subset of pituitary tumours with constitutively active adenylyl cyclase<sup>8</sup> carry oncogenic mutations in the  $\alpha_s$  gene. Moreover, the biochemical properties and cellular function of  $\alpha_s$ —unlike the products of most oncogenes—have been characterized in detail. For this reason, the locations and functional alterations produced by these tumour-associated  $\alpha_s$  mutations significantly extend our understanding of the conserved mechanism of GTP hydrolysis in the signalling G proteins, p21<sup>ras</sup> and many other GTP-binding proteins. Specifically, they point to a region of  $\alpha_s$  that is intimately involved in GTP hydrolysis and that probably acts in  $\alpha_s$  as a built-in counterpart of the separate GTPase-activating proteins (GAPs)<sup>9,11</sup> required for GTP hydrolysis by other GTP binding proteins, such as p21<sup>ras</sup>.

GH-releasing hormone (GHRH) uses cAMP as a second messenger to stimulate GH secretion and proliferation of normal pituitary somatotrophs<sup>11</sup>. Vallar *et al.*<sup>8</sup> reported constitutive activation of  $G_s$  in a subset of GH-secreting human pituitary tumours. Measurements of adenylyl cyclase activity clearly identified two distinct groups of tumours: group 1 tumours had low

basal adenylyl cyclase activity that responded normally to stimulation by hydrolysis-resistant GTP analogues, AIF<sub>4</sub><sup>−</sup> ion and GHRH, whereas group 2 tumours had marked elevation of basal adenylyl cyclase and responded poorly or not at all to the stimulatory agents.  $G_s$  activity was normal in group 1 tumours and constitutively active in group 2 tumours<sup>8</sup>.

Prompted by the idea that  $\alpha_s$  activated by mutation could relieve the requirement for GHRH stimulation of adenylyl cyclase and thereby contribute to growth of these tumours, we sought and found mutations in  $\alpha_s$  genes of group 2 tumours.

## Identification of $\alpha_s$ mutations

We tested eight pituitary tumours surgically removed from acromegalic patients suffering from excessive secretion of GH. Using basal adenylyl cyclase activity and its response to stimulation by AIF<sub>4</sub><sup>−</sup> as criteria, we identified four group 1 tumours and four in group 2 (Table 1). As assessed by biochemical complementation of  $\alpha_s$ -deficient S49 *cyc*<sup>−</sup> membranes<sup>8</sup>,  $G_s$  activity was constitutively elevated (in the presence of GTP alone) in the four group 2 tumours and normal in the four group 1 tumours (results not shown).

To look for mutations in the group 2 tumours, we reverse-transcribed RNA, amplified the coding region of  $\alpha_s$  complementary DNA using the polymerase chain reaction (PCR)<sup>12</sup>, subcloned the amplified DNA into M13 vectors, and sequenced the entire coding region from multiple M13 clones derived from each tumour. The  $\alpha_s$  cDNAs from all four group 2 tumours contained mutations (Table 1). Mutations in three tumours replaced Arg 201 (wild-type codon CGT) with either Cys (TGT in tumours 5 and 7) or His (CAT in tumour 6); using the single-letter code for amino acids, we designate these mutations R201C and R201H, respectively. The mutation in tumour 8 replaced Gln 227 (wild-type codon CAG) with Arg (CGG); this mutation is designated Q227R.

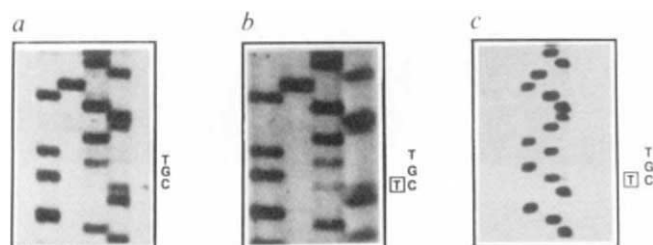


FIG. 1 Direct sequencing of PCR-amplified region surrounding codon 201. a, Genomic DNA from tumour 1 (group 1). b, Genomic DNA from tumour 5 (group 2). c, cDNA from tumour 5 (group 2). Lanes from left to right are G, A, T, C. The nucleotide sequence of codon 201 is listed beside each panel. The mutant nucleotide in tumour 5 is boxed. Direct sequencing was performed as described in Table 1 legend.

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TABLE 1  $\alpha_s$  Mutations in human pituitary tumours

| Tumour  |   | Adenylyl cyclase<br>(pmol cAMP mg <sup>-1</sup> min <sup>-1</sup> ) |                  | DNA             | Codon 201       | Codon 227       |
|---------|---|---------------------------------------------------------------------|------------------|-----------------|-----------------|-----------------|
|         |   | Basal                                                               | AlF <sub>4</sub> |                 |                 |                 |
| Group 1 | 1 | 13                                                                  | 170              | Genomic         | Arg             | Gln             |
|         | 2 | 6                                                                   | 96               | Genomic         | Arg             | Gln             |
|         | 3 | 16                                                                  | 300              | Genomic         | Arg             | Gln             |
|         | 4 | 43                                                                  | 130              | Genomic         | Arg             | Gln             |
| Group 2 | 5 | 170                                                                 | 130              | cDNA            | Arg (2)/Cys (3) | Gln             |
|         |   |                                                                     |                  | Genomic         | Arg/Cys         | Gln             |
|         | 6 | 480                                                                 | 260              | cDNA            | Arg (0)/His (4) | Gln             |
|         |   |                                                                     |                  | Genomic         | Arg/His         | Gln             |
|         |   |                                                                     |                  | Genomic (blood) | Arg             | Gln             |
|         | 7 | 190                                                                 | 130              | cDNA            | Arg (0)/Cys (3) | Gln             |
|         |   |                                                                     |                  | Genomic         | Arg/Cys         | Gln             |
|         | 8 | 180                                                                 | 120              | cDNA            | Arg             | Gln (0)/Arg (3) |
|         |   |                                                                     |                  | Genomic         | Arg             | Gln/Arg         |
|         |   |                                                                     |                  | Genomic (blood) | Arg             | Gln             |

Eight pituitary tumours are divided into groups 1 and 2 by adenylyl cyclase activities. Columns on right list, for each tumour, the source of DNA (genomic DNA from tumour, cDNA from tumour, or genomic DNA from peripheral blood of the same patient) and the amino acid(s) encoded by codons 201 and 227, determined by sequencing PCR-amplified cDNA or genomic DNA. Two amino acids are listed when bases encoding both were found in a single DNA sample. Numbers in parentheses indicate the number of individual M13 cDNA clones sequenced that encoded the specified amino acid. Pituitary adenomas from patients with GH excess were surgically removed and stored at  $-70^{\circ}\text{C}$ . To measure adenylyl cyclase activity, membranes were prepared from tumour homogenates and adenylyl cyclase activities measured<sup>8</sup> in the absence or presence of 10 mM NaF. To perform sequence analysis of subcloned PCR amplified cDNA, total RNA was extracted from tumour homogenates as described<sup>36</sup>. First strand cDNA was produced in a reaction containing 7  $\mu\text{g}$  total RNA, oligo(dT) primer and reverse transcriptase, according to the protocol in the AMV reverse transcriptase kit from Bethesda Research Laboratories. Four per cent of the cDNA reaction volume was amplified as described<sup>12</sup>. Primers were designed to match the 5' and 3' noncoding regions of human  $\alpha_s$  (ref. 37), in order to amplify the complete coding region. Each primer contained an artificial restriction site at its 5' end to facilitate subcloning. The 5' primer (5'-GCCGGTACCCGGCCGCCGCCGCCGCCG-3') had a *KpnI* restriction site and the 3' primer (5'-TTAAAGCTTTAATTAAATTTGGGGTTCC-3') had a *HindIII* restriction site. The PCR mixture contained 2.5 U DNA polymerase from *Thermus aquaticus* (Perkin-Elmer Cetus) and 25 pmol of each primer. Amplification was accomplished in 40 cycles of 1 min at  $94^{\circ}\text{C}$ , 1 min at  $58^{\circ}\text{C}$ , and 2 min at  $72^{\circ}\text{C}$ , in a Perkin-Elmer Cetus Thermocycler. Amplified DNA was desalted and primers were removed by gel filtration with G-50 Sephadex spin columns (Boehringer Mannheim) and ethanol precipitation. The purified DNA was digested with *KpnI* and *HindIII* and subcloned into M13mp18 or M13mp19. The Sequenase method of dideoxy sequencing was used to determine the entire  $\alpha_s$  coding sequence from at least two M13 clones derived from two separate amplification reactions for each group 2 tumour. For direct sequence analysis of PCR amplified cDNA and genomic DNA, first strand cDNA from tumours was prepared as described above. Genomic DNA was extracted from tumours or peripheral blood by homogenizing samples in a glass-Teflon homogenizer in a lysis buffer (4 M Urea, 1% Triton X-100, 10 mM EDTA, 100 mM NaCl, 10 mM Tris, pH 8.0, 10 mM DTT, and 0.2 mg ml<sup>-1</sup> Proteinase K); samples were incubated overnight, repeatedly extracted with phenol/chloroform, and DNA recovered by ethanol precipitation. PCR amplification was performed with primers flanking a region that encompassed both codons 201 and 227; the 5' primer (5'-GTGATCAAGCAGGCTGACTATGTG-3') was located in exon 7 and the 3' primer (5'-GCTGTGTCGCCACCACGAAGATGAT-3') was located in exon 10 (ref. 37). Four per cent of the cDNA reaction volume or 50 ng genomic DNA was amplified as described above, except that unequal molar concentrations (12.5 pmol:1 pmol) of the primers were used to generate single-stranded DNA for direct sequence analysis, as described<sup>38</sup>. The reaction mixture was desalted and excess dNTPs were removed by repeated (3–4  $\times$ ) spin-dialysis on a Centricon 30 (Amicon). Samples were then vacuum-dried and resuspended in water. Half of the reaction product was sequenced using Sequenase. The primer used at low concentration in the amplification reaction was used in the sequencing reaction.

Both amino-acid residues replaced by these mutations were already known or suspected to be important in  $\alpha_s$  function. Arg 201 is the residue thought to be ADP-ribosylated by the pathogenic exotoxin of *Vibrio cholerae*<sup>13</sup>, a covalent modification that activates  $\alpha_s$  by inhibiting its GTPase activity<sup>14</sup>. Gln 227 is located in the presumed guanine nucleotide binding region of  $\alpha_s$ <sup>15</sup>, at a position corresponding to Gln 61 of p21<sup>ras</sup>; mutational replacement of Gln 61 in p21<sup>ras</sup> by almost any other amino acid produces a protein that promotes malignant transformation<sup>16</sup>. The functional significance of mutations at these two sites is discussed in more detail below.

To confirm and extend these findings, we determined nucleotide sequences surrounding codons 201 and 227 by direct sequencing of PCR-amplified regions of genomic  $\alpha_s$  DNA (see Table 1 legend for details). Genomic DNA from each of the group 1 tumours showed only wild-type sequence at codons 201 and 227. Genomic DNA from each of the group 2 tumours showed the same mutation detected in the corresponding cDNA. Group 2 genomic samples also showed wild-type sequence at the same positions (example in Fig. 1; summarized in Table 1). This presumably indicates the simultaneous presence of both a mutant and a normal  $\alpha_s$  allele and suggests that constitutive activation of  $\alpha_s$  is a dominant phenotype (see below). By analogy

with most dominant oncogenes, we expect the  $\alpha_s$  mutations to be somatic in origin and, in keeping with this expectation, genomic DNA from peripheral blood cells of two patients with group 2 tumours contained only wide-type  $\alpha_s$  sequences (Table 1).

### Mutations constitutively activate $\alpha_s$

To determine whether the mutations cause constitutive activation of  $\alpha_s$  and how they might do so, we altered the nucleotide sequence at appropriate codons of a cDNA encoding a form of rat  $\alpha_s$  of relative molecular mass 52,000 (ref. 17) and expressed the R201C, R201H, and Q227R mutant proteins in  $\alpha_s$ -deficient S49 *cyc*<sup>-</sup> cells<sup>18–21</sup> (for details, see Fig. 2 legend). The adenylyl cyclase phenotypes of cells expressing the mutant proteins were compared with that of a clone (csw1.3) of *cyc*<sup>-</sup> cells transformed with wild-type rat  $\alpha_s$  (refs 18, 20 and 21).

In *cyc*<sup>-</sup> cells, each of the three mutant proteins reproduced the adenylyl cyclase phenotype observed in group 2 tumours. As compared with wild-type  $\alpha_s$  (in csw1.3 membranes), adenylyl cyclase in membranes of Q227R, R201C, and R201H cells showed elevated basal activity (measured in the presence of GTP alone) (Fig. 2). Relative to GTP alone, GTP plus isoprenaline, a  $\beta$ -adrenoceptor agonist, stimulated a substantially smaller



increase in adenylyl cyclase in membranes containing mutant  $\alpha_s$  (2- to 3-fold) than in csw1.3 membranes (13-fold).  $\text{AlF}_4^-$  ion and GTP- $\gamma$ S, a hydrolysis-resistant GTP analogue, stimulated adenylyl cyclase in csw1.3 membranes, but not in Q227R, R201C, or R201H membranes (Fig. 2).

These results closely parallel patterns of adenylyl cyclase responsiveness observed in membranes of group 2 tumours, which converted ATP to cAMP at a markedly elevated rate in the absence of stimulatory agents. Adenylyl cyclase in these tumour membranes was stimulated only slightly by GHRH and not at all by GTP analogues or  $\text{AlF}_4^-$  (ref. 8).

In principle, the Gln 227 or Arg 201 mutations could activate  $\alpha_s$  in two ways. Either type of mutation could mimic the normal effect of hormone-receptor complex, which activates  $\alpha_s$  by increasing the rate at which GDP dissociates from its inactive form, thereby accelerating binding of GTP and attainment of the active state. Alternatively, mutations could inhibit the intrinsic GTPase of  $\alpha_s$ , which serves as a timing device that turns off the protein's active GTP-bound conformation<sup>3-6</sup>. This second mechanism turns out to account for  $\alpha_s$  activation by mutations at both sites, as described below.

### Gln 227 mutations

Even before the Q227R mutation was identified, site-directed mutations<sup>21,22</sup> had shown that mutational replacement of Gln 227 by Leu (Q227L) activates  $\alpha_s$  by inhibiting its GTPase activity. Similarities in primary structure between short stretches of conserved amino-acid sequence in G protein  $\alpha$ -chains and parts of the guanine nucleotide-binding domain of p21<sup>ras</sup> had suggested<sup>15,23</sup> that these  $\alpha$ -chain regions form a GTP-binding domain structurally and functionally similar to that of p21<sup>ras</sup>. The similar effects of the  $\alpha_s$  Q227L mutation and the cognate Q61L *ras* mutation on GTPase, and biological activity of the

respective mutant proteins<sup>16,24</sup> confirmed that  $\alpha$ -chains and p21<sup>ras</sup> share similar GTP-binding domains.

Accordingly, we examined the effect on GTPase activity of the tumour-associated  $\alpha_s$ -Q227R mutation expressed in *cyc*<sup>-</sup> cells. The Q227R mutation inhibited GTPase activity of  $\alpha_s$ , reducing  $k_{\text{cat-GTP}}$  from a wild-type value of 4.1 min<sup>-1</sup> to 0.12 min<sup>-1</sup> (Fig. 3). These  $k_{\text{cat-GTP}}$  values were estimated by an indirect method<sup>25</sup> based on measurements of membrane-associated adenylyl cyclase activity (Fig. 3). The genetically engineered Q227L mutation similarly reduced  $k_{\text{cat-GTP}}$  measured by both the indirect method<sup>21</sup> and by direct assay using pure recombinant protein expressed in *Escherichia coli*<sup>22</sup>. The very similar results obtained by the two methods<sup>21,22</sup> support the validity of the indirect method for estimating  $k_{\text{cat-GTP}}$  of  $\alpha_s$ .

Thus phenotypes produced by the deliberately designed Q227L mutation and by the Q227R mutation found in a human pituitary tumour are consistent with the hypothesis that  $\alpha_s$  and p21<sup>ras</sup> share structurally similar guanine nucleotide-binding domains and use similar or identical mechanisms to hydrolyse GTP.

### Arg 201 mutations inhibit GTPase

Although they lack precedents in p21<sup>ras</sup> or other GTP-binding proteins, the Arg 201 mutations yield potentially important clues regarding the mechanism of GTP hydrolysis by  $\alpha_s$ . In keeping with their effects on GTP-dependent adenylyl cyclase activity (Fig. 2), the R201C and R201H mutations inhibited GTPase activity of  $\alpha_s$ , reducing  $k_{\text{cat-GTP}}$  ~30-fold to 0.12 min<sup>-1</sup> and 0.15 min<sup>-1</sup>, respectively (Fig. 3).

Because Arg 201 is the  $\alpha_s$  residue whose side chain is thought to be ADP-ribosylated by the exotoxin of *V. cholerae*, we also measured the  $k_{\text{cat-GTP}}$  of wild-type  $\alpha_s$  in membranes treated with cholera toxin and NAD<sup>+</sup> (Fig. 3). Toxin treatment decreased  $k_{\text{cat-GTP}}$  from 4.1 to ~0.17 min<sup>-1</sup>. The effect of toxin-catalysed ADP-ribosylation of Arg 201 closely resembles the effects of replacing the same residue by Cys or His. This similarity indicates that attachment of ADP-ribose to Arg 201 inhibits GTPase more specifically than expected: perhaps the rate of GTP hydrolysis is not reduced by nonspecific steric hindrance from the bulky ADP-ribose attached to Arg 201, but rather because the specific shape and location of the Arg 201 side chain itself are necessary for normal GTP hydrolysis.

This notion predicts that replacement of Arg 201 by virtually any amino acid will inhibit GTPase. Soon after finding the R201C and R201H mutations in pituitary tumours, we learned that this prediction had already been partially fulfilled; Freissmuth and Gilman constructed recombinant mutant  $\alpha_s$  in which Arg 201 was replaced by Ala, Glu or Lys, and in each case the recombinant mutant  $\alpha_s$  protein, synthesized in *E. coli*, showed markedly decreased  $k_{\text{cat-GTP}}$  and enhanced ability to stimulate adenylyl cyclase in the presence of GTP alone (A. G. Gilman, personal communication). Duplication of the functional change by replacing Arg 201 with five different amino acids makes it quite likely that the side chain of this Arg residue interacts with or forms a part of the molecular timing device that regulates  $k_{\text{cat-GTP}}$ . The fact that substitution of a positively charged Lys residue for Arg 201 inhibits GTPase indicates, in addition, that the postulated function of Arg 201 cannot be supplied simply by a side chain with positive electrical charge.

Activated cholera toxin is a mono-ADP-ribosyltransferase, which transfers ADP-ribose from NAD<sup>+</sup> to the side chain of arginine and arginine methyl ester, but not to other amino acids<sup>26</sup>. Consequently, we expected that the toxin would not ADP-ribosylate  $\alpha_s$ -R201C or  $\alpha_s$ -R201H. This prediction was not completely fulfilled: the toxin catalysed transfer of radiolabel from [<sup>32</sup>P]NAD<sup>+</sup> to both  $\alpha_s$ -R201C and  $\alpha_s$ -R201H expressed in *cyc*<sup>-</sup> cells, although much less efficiently than seen with wild-type  $\alpha_s$  (Fig. 4). Although precise estimates of the relative extent of ADP-ribosylation are difficult, owing to differing amounts of  $\alpha_s$  protein in different membrane preparations, we estimated that

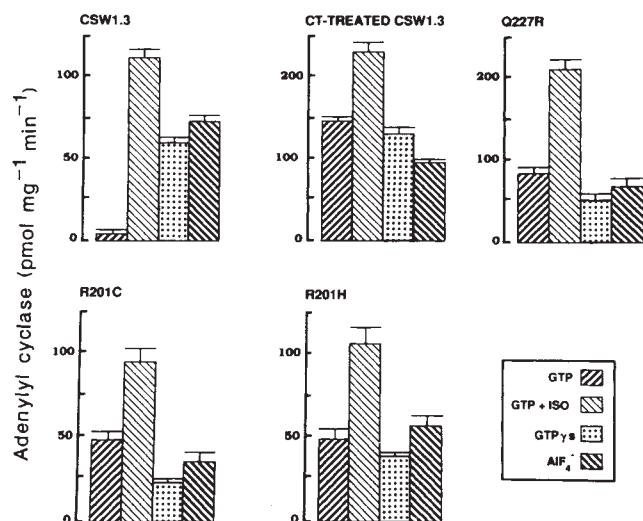


FIG. 2 Adenylyl cyclase stimulated by normal and mutant  $\alpha_s$ . Membranes were assayed for adenylyl cyclase activity in the presence of the following concentrations of activators: 50  $\mu$ M GTP; 100  $\mu$ M isoprenaline (ISO); 50  $\mu$ M GTP- $\gamma$ S; 10  $\mu$ M  $\text{AlCl}_3$  + 10 mM NaF ( $\text{AlF}_4^-$ ). Values represent means  $\pm$  s.d. for triplicate determinations.

METHOD. The Q227R, R201C and R201H mutations were introduced into the 52 K form of a normal rat  $\alpha_s$  cDNA<sup>17,21</sup> by site-directed mutagenesis with the Bio-Rad Muta-Gene Phagemid *in vitro* mutagenesis kit<sup>39</sup>. Mutant  $\alpha_s$  DNAs were subcloned into the retroviral expression vector MV7 and transfected into S49 *cyc*<sup>-</sup> cells as described previously<sup>18,20,21</sup>. Membranes were prepared from *cyc*<sup>-</sup> clones that expressed the mutant  $\alpha_s$  proteins<sup>21</sup>. Cholera toxin treatment of membranes and adenylyl cyclase assays were performed as described previously<sup>21</sup>, except that the time of incubation in the adenylyl cyclase assays was decreased from 30 to 15 min.

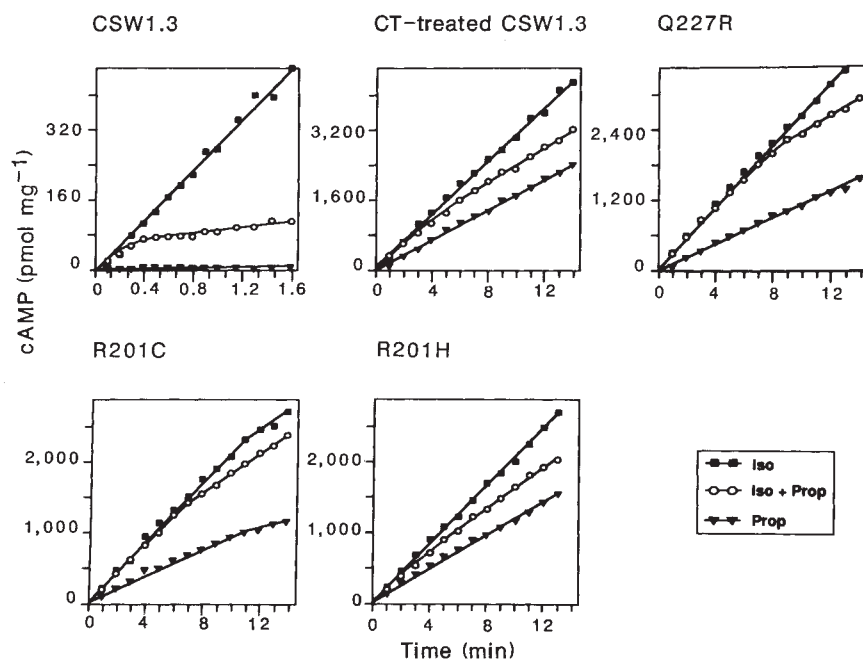


FIG. 3 Indirect measurement of GTPase. The average duration of 'active'  $G_s$  was assessed indirectly, by adaptation<sup>21</sup> of a method<sup>25</sup> using measurements of adenylyl cyclase activity. Briefly, adenylyl cyclase in membranes was first activated for 3 min by isoprenaline (20  $\mu$ M) plus GTP (50  $\mu$ M) in the presence of non-radioactive ATP; at time = 0, agonist-promoted introduction of GTP into the binding site of  $G_s$  was arrested by addition of a  $\beta$ -adrenoceptor antagonist, propranolol (50  $\mu$ M). [ $\alpha$ -<sup>32</sup>P]ATP was added simultaneously with propranolol, and aliquots were removed at the indicated times for determination of [<sup>32</sup>P]cAMP (see Fig. 2 legend). The rate constant for the GTPase turn-off reaction,  $k_{cat-GTP}$ , was calculated from the time course (Iso + Prop,  $\circ$ ) of the propranolol-induced reduction in the rate of agonist-dependent cAMP synthesis (down to the rate measured in the absence of agonist (Prop,  $\blacktriangledown$ )). Also shown (Iso,  $\blacksquare$ ) is the time course of isoprenaline-stimulated [<sup>32</sup>P]cAMP synthesis in the absence of propranolol.

the toxin labelled  $\alpha_s$ -R201C < 10% as efficiently as wild-type  $\alpha_s$ , and  $\alpha_s$ -R201H 10–20% as efficiently as wild type  $\alpha_s$  (see Fig. 4 legend for details). M. Freissmuth and A. G. Gilman (personal communication) found similar results with their mutants: in each case, the toxin ADP-ribosylated R201X mutant  $\alpha_s$  < 20% as efficiently as it modified wild type  $\alpha_s$ .

This unexpected set of results can be explained in at least two ways. First, Arg 201 could be mistakenly identified as the amino acid modified by cholera toxin, which may normally ADP-ribosylate another Arg residue. Arg 201 of  $\alpha_s$  has not been directly shown to be modified by the toxin. Instead, the direct experiment<sup>13</sup> was performed with  $\alpha_i$ , the  $\alpha$ -subunit of retinal transducin:  $\alpha_i$  was modified by the toxin at Arg 174, which corresponds to Arg 201 of  $\alpha_s$ . A second interpretation, perhaps more likely, is that the toxin's ADP-ribosyltransferase activity prefers Arg 201 but will use another residue as the acceptor when confronted with an  $\alpha_s$  molecule lacking an Arg side chain at the correct position. In this context it is interesting that  $\alpha_s$  (like almost all other G protein  $\alpha$ -chains) contains another Arg residue just two positions upstream from Arg 201; perhaps this residue serves as a secondary acceptor site for ADP-ribose.

In keeping with the relatively low extent of toxin-catalysed modification of  $\alpha_s$ -R201C and  $\alpha_s$ -R201H, treatment with toxin and NAD<sup>+</sup> did not alter the ratio of adenylyl cyclase activities measured in the presence of GTP compared with GTP plus isoprenaline (result not shown).

### Conditional versus intrinsic GTPases

Arg 201 is located in domain II (ref. 15) of  $\alpha_s$  (residues 61–218). This domain is demarcated at either end by conserved signature sequences (GX<sup>5</sup>XXGK and DX<sup>5</sup>XGQ) that are found in the guanine nucleotide domains of other GTP-binding proteins, such as p21<sup>ras</sup> and GTPases involved in ribosomal protein synthesis, exemplified by elongation factor Tu (EF-Tu). The function of the 158 residues of domain II and of the cognate domains of similar size in other G protein  $\alpha$ -chains, is unknown. Domain II has no obvious counterparts in p21<sup>ras</sup> and EF-Tu, in which the topologically corresponding regions are very much shorter (29 and 47 residues, respectively)<sup>15</sup>. Consequently it is reasonable to speculate that domain II may perform a function that is lacking in p21<sup>ras</sup> and EF-Tu.

The high intrinsic rate of GTP hydrolysis by G proteins represents one such function. The signalling G proteins hydrolyse GTP at an unvarying intrinsic rate, whereas p21<sup>ras</sup> and

EF-Tu hydrolyse GTP at rates that crucially depend on the presence of other proteins. The  $k_{cat}$  for GTP hydrolysis by G proteins is relatively fast (> 1 min<sup>-1</sup>) and unaffected by binding to other proteins<sup>21,22,27</sup>. By contrast, p21<sup>ras</sup> and EF-Tu hydrolyse GTP extremely slowly ( $k_{cat-GTP}$  values of 0.02 min<sup>-1</sup> or even lower<sup>28,29</sup>) unless their GTPase is stimulated by other proteins—GTPase-activating protein<sup>9,10</sup> in the case of p21<sup>ras</sup> and the programmed ribosome in the case of EF-Tu (ref. 29).

Taken together, the parallel structural and functional differences raise the intriguing possibility that domain II serves as an intrinsic, 'built-in' GAP for G protein  $\alpha$ -chains. This idea provides tentative explanations both for the large 'excess' sequence in domain II of G proteins relative to other GTP-binding proteins, and for the location of this excess sequence in  $\alpha$ -chains; the topological equivalent of domain II in p21<sup>ras</sup> contains the so-called 'effector' region (residues 33–40), in which mutational replacements of amino acids prevent interaction with GAP<sup>10,30,31</sup>. Thus the location of the postulated intrinsic GAP activity in the primary structure of G protein  $\alpha$ -chains roughly coincides with the location of p21<sup>ras</sup> residues which interact with a separate and distinct GAP protein; perhaps this coincidence implies similarly cognate locations of the two kinds of GAP in three dimensions as well. In this view, Arg 201 and cognate Arg residues in other G protein  $\alpha$ -chains play key roles in the interaction between the intrinsic GAP-like domain and the GTP-binding domain. Mutations that replace Arg 201 or bacterial toxins that modify its side chain inhibit GTPase activity by interrupting this interaction. Although highly speculative, the postulated GAP-like function of domain II can be readily tested.

### Is $\alpha_s$ an oncogene protein?

Are the mutant  $\alpha_s$  proteins in group 2 pituitary tumours products of oncogenes? They resemble previously discovered dominantly acting oncogene proteins in four respects: (1) The mutant proteins mimic the effect of an extracellular growth factor—in this case, GHRH, which stimulates proliferation of normal GH-secreting pituitary cells by stimulating adenylyl cyclase<sup>11</sup>. (2) The mutations stabilize the active conformation of a signalling protein in a pathway that normally stimulates growth of the cell type carrying the putative oncogene. (3) The  $\alpha_s$  mutations are found in tumour DNA but not in DNA from other cells (in this case, peripheral blood) of the same patients (Table 1). (4) The tumour genome contains both mutant and non-mutant



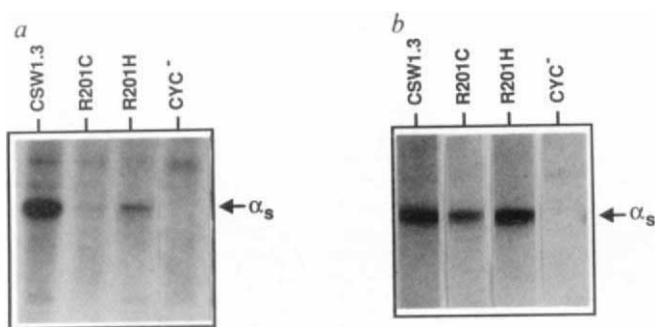


Fig. 4 Cholera toxin labelling and immunoblot analysis of wild-type and mutant  $\alpha_s$ . *a*, The transfer of [ $^{32}$ P]NAD to  $\alpha_s$  proteins by treatment of membranes (100  $\mu$ g membrane protein) with cholera toxin was performed as described previously<sup>21</sup>. Labelled proteins were resolved on a 10% SDS polyacrylamide gel<sup>21</sup>. The labelling of R201X mutants, relative to labelling of the wild-type protein in csw1.3 membranes, was estimated by densitometric analysis of autoradiographs, using a LKB Ultrascan XL densitometer, and these values were corrected by the levels of  $\alpha_s$  expression (see below). These calculations indicated that the toxin labelled R201X proteins much less efficiently than wild type  $\alpha_s$  (<10% as well for R201C and 10–20% as well for R201H). *b*, To estimate the amount of wild-type or mutant  $\alpha_s$  expressed in the *cyc*<sup>-</sup> clones, membrane proteins (90  $\mu$ g csw1.3, R201C, and *cyc*<sup>-</sup>; 70  $\mu$ g R201H) were resolved on a 10% SDS-polyacrylamide gel, transferred to nitrocellulose and immunoblotted<sup>20,21</sup> with affinity-purified antiserum directed against a peptide located in the carboxy-terminal half of  $\alpha_s$ . Relative amounts of  $\alpha_s$  were determined by densitometric analysis of autoradiographs and a standard curve constructed from blots of varying amounts of csw1.3 membranes (0–90  $\mu$ g protein per lane). Estimates of these amounts, expressed as percentages of that in csw1.3 membranes (100%), were 34% in R201C (range = 31–38%, *n* = 3) and 115% in R201H (range = 89–149%, *n* = 4).

copies of the putative oncogene, indicating—as would be expected from the GTPase defect—that the effect of the mutant  $\alpha_s$  is dominant with respect to wild-type  $\alpha_s$ .

We have confirmed that a constitutively active  $\alpha_s$  protein can produce dominant biochemical effects in tissue culture: expression of  $\alpha_s$ -Q227L in Swiss-3T3 cells elevates cellular cAMP and augments DNA synthesis in serum-depleted medium containing insulin (I. Zachary, S.B.M. and H.R.B., manuscript in preparation). The straightforward implication—that the Q227 and R201  $\alpha_s$  mutations should produce similarly dominant

effects on cAMP synthesis and proliferation of pituitary somatotrophs—must be qualified, however. Several findings hint that mutant  $\alpha_s$  transcripts outnumber wild-type transcripts in group 2 tumours. Sequencing gels prepared from PCR-amplified genomic tumour DNA revealed wild-type and mutant bases at roughly equal intensities, indicating equal numbers of wild-type and mutant  $\alpha_s$  genes. By contrast, direct sequencing of PCR-amplified cDNA from the same tumours invariably showed a more prominent signal from the mutant base (see Fig. 1). This is indicative of a preponderance of mutant  $\alpha_s$  transcripts in group 2 tumours, which is corroborated by the disproportionately large number of mutant  $\alpha_s$  cDNAs in M13 subclones (13 out of 15 sequenced; Table 1). Further investigation is required to determine whether expression of the mutant  $\alpha_s$  genes in these tumours quantitatively exceeds that of wild-type  $\alpha_s$  genes and, if so, to determine the mechanism responsible. At this stage we suggest that the biochemical dominance of GTPase-deficient  $\alpha_s$  may be augmented by a second defect, so far not identified, that increases relative expression of the mutant  $\alpha_s$  gene. Increased relative expression of dominant mutant oncogenes is often found in tumours, presumably because it confers a selective proliferative advantage on the tumour cell (for a summary, see ref. 32).

The most stringent criterion for qualification as an oncogene is that deliberate expression of the mutant gene should reproducibly induce formation of tumours. It is important to recognize that the postulated tumorigenic effect of mutant  $\alpha_s$  would be exerted only in a relatively small subset of differentiated cells, those programmed to proliferate in response to elevated cAMP; this subset includes several endocrine target tissues (such as ovary, adrenal cortex, thyroid and some cells of the pituitary) and a small number of other cell types<sup>1,2</sup>. For this reason, stringent tests of the  $\alpha_s$  oncogene hypothesis may require cell- or tissue-specific expression of mutant  $\alpha_s$  genes in transgenic mice. In anticipation that such tests will be positive, we suggest that the postulated oncogene be designated *gsp*, for G<sub>s</sub> protein.

Finally, it is worth noting that other G protein  $\alpha$ -chains share with  $\alpha_s$  a common mechanism for binding and hydrolysing GTP, as well as highly conserved primary structure in regions that correspond to the locations of Arg 201 and Gln 227 of  $\alpha_s$ . Mutational replacement of these cognate residues in certain other  $\alpha$ -chains will probably inhibit GTPase and generate constitutively active signals, such as those of the phosphoinositide/Ca<sup>2+</sup> cascade. For this reason, we predict that new oncogenes will result from mutations in genes that encode the  $\alpha$ -chains of G<sub>i</sub>, G<sub>o</sub> (refs 3, 4, 6, 33) and G<sub>z</sub> (refs 34, 35). □

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# Far-infrared observations of thermal dust emission from supernova 1987A

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**INFRARED observations of supernova 1987A are important for studying both the evolution of the ejecta and its interaction with the surrounding interstellar medium. Here we report observations of SN1987A, in the spectral range 18–35  $\mu\text{m}$ , taken on 16 and 23 November 1988, 632 and 639 days after core collapse. A strong ( $\sim 10$  Jy at 20  $\mu\text{m}$ ) and rather flat continuum underlies weak fine-structure lines from heavy elements, and declines slowly between 24 and 30  $\mu\text{m}$ . Its spectral shape is indicative of thermal emission from an almost featureless dust component, probably graphite, with silicates contributing <20% of the emitting dust mass. Some of the emission may be an 'echo' of supernova light reflected from a pre-existing dust cloud<sup>1</sup>, but a better explanation, which can account for the entirety of emission from infrared to gamma wavelengths, is that dust is being formed in the supernova ejecta<sup>2</sup>. This also accounts more naturally for the inferred dust composition. Continuous observation is needed to determine the relative importance of these two components of the infrared emission.**

Roche *et al.*<sup>1</sup> have found spatially extended emission at 10- $\mu\text{m}$  wavelength which they attribute to a thermal infrared echo from a pre-existing dust cloud. If this cloud is responsible for the emissions we observe, we would also expect to see other effects, such as an inflection in the visual-light curve of the supernova, as the light from SN1987A is scattered off the dust cloud, as well as diffuse light around the supernova<sup>3</sup>. Both effects have been reported<sup>3–6</sup>, but at a lower flux level than model calculations give, suggesting that only some fraction of the infrared light is

an echo. At the same time, measurements of asymmetries in the O I and C I emission-line profiles<sup>2</sup> are evidence for the existence of some newly formed dust in the supernova ejecta.

The infrared observations of SN1987A were obtained on two flights with the Kuiper Airborne Observatory (KAO) on 16 and 23 November 1988. Details of the observations, the calibration, data reduction techniques and discussion of the fine-structure lines in our data will be presented in a separate paper (S.H.M., E.D., W.G., J.R.G., R.F.L. and R.F.S., manuscript in preparation). Here we concentrate on the continuum emission, shown in Fig. 1 together with selected data points from the 8–13  $\mu\text{m}$  observations<sup>1</sup> that were obtained on 25 September (day 580 after core collapse). The spectrum shows the presence of weak fine-structure lines at 17.9  $\mu\text{m}$  and at wavelengths  $>23$   $\mu\text{m}$ . The broad structure seen in the 19–23  $\mu\text{m}$  region is still unidentified, and could be part of the underlying continuum emission. The continuum level is quite strong,  $\sim 10$  Jy at 20  $\mu\text{m}$ , and at the same level as the 8–13  $\mu\text{m}$  observations on day 580. (The increase in the continuum at about day 450 (ref. 1) after a steady decrease from about day 120 and the steady decrease in the ionization state of the ejecta (ref. 1; S.H.M., E.D., W.G., J.R.G., R.F.L. and R.F.S., manuscript in preparation), suggest a thermal dust origin (instead of free-free radiation) for the emission. Furthermore, the smoothness of the spectrum limits the amount of silicate dust<sup>3</sup> (which has strong emission features at 9.7 and 18  $\mu\text{m}$ ) contributing to the emission.

Roche *et al.*<sup>1</sup> suggested that the emission is an infrared echo from a dust cloud located at a distance of about one light year behind the supernova, instead of dust that has recently formed in the expanding supernova ejecta. To calculate the mass and temperature of radiating dust, we constructed a simple dust model consisting of a population of graphite particles characterized by a single grain radius of 0.1  $\mu\text{m}$ . We used graphite optical constants from ref. 7. Figure 1 shows the spectra of 300, 350 and 400 K graphite grains, normalized to produce the best visual fit to a lower envelope of continuum emission. The ambiguity in the dust temperature is due to the fact that the 8–13  $\mu\text{m}$  data were not taken at the same time as the KAO observations were made, and the fluxes on day 635 could be higher or lower. A dust temperature of 400 K will require the 10- $\mu\text{m}$  flux to be still rising on day 580, whereas a dust temperature of 300 K implies that it had already peaked before that time. At temperatures between 300–400 K the infrared spectrum peaks around 10  $\mu\text{m}$ , and the relative flatness of the spectrum at longer wavelengths is a result of the enhanced emissivity of graphite (over that

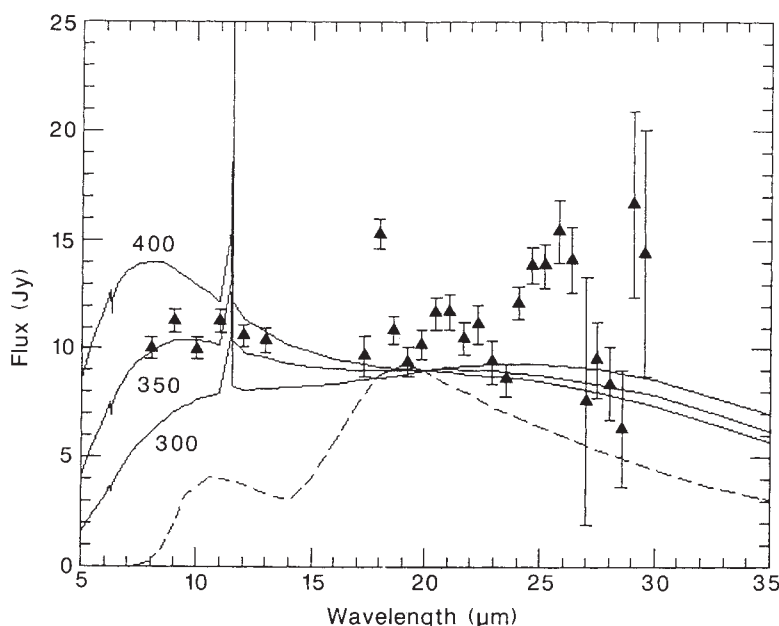


FIG. 1 The 18–35  $\mu\text{m}$  spectrum of SN1987A obtained with the KAO on days 632 and 638 after core collapse, shown together with selected points from the 8–13  $\mu\text{m}$  observations of Roche *et al.*<sup>1</sup>. The elevated points (triangles) in the 18–35  $\mu\text{m}$  spectrum are due to emission from fine-structure lines of Fe I, Si and Fe II (S.H.M., E.D., W.G., J.R.G., R.F.L. and R.F.S., manuscript in preparation). The solid lines represent the infrared spectrum of a population of 0.1- $\mu\text{m}$  graphite particles radiating at temperatures of 300, 350 and 400 K. Their spectrum was normalized to give the best visual fit to the underlying continuum emission in the 18–29  $\mu\text{m}$  wavelength regime. Silicates of the same size and distance as the 400 K graphite dust will be heated to only  $\sim 180$  K. Their spectrum (arbitrarily normalized to 9 Jy at 20  $\mu\text{m}$ ) is also shown (dashed line).



obtained by extrapolating the 10- $\mu\text{m}$  value with a  $\lambda^{-1}$  emissivity law) in the  $\sim 20$ - $\mu\text{m}$  wavelength regime. At 100  $\mu\text{m}$  the infrared flux from these single-temperature dust models is  $<1$  Jy. Our choice of graphite was motivated by the smoothness of the infrared spectrum. Amorphous carbon has a smooth spectrum as well, but it is not as broad as that of graphite, and the wide range of dust temperatures required to fit the observations with amorphous carbon might be hard to justify on theoretical grounds.

Some limits on the dust temperature may be set using the spatial extent of the emission<sup>1</sup> full-width half-maximum (FWHM) of 1.5 arcsec on day 580 and the echo delay time<sup>3</sup>. For dust temperatures of 300 and 350 K the model presented in ref. 3 shows that the projected radii of the radiating cloud will be 2.1 arcsec and 1.5 arcsec, respectively, which are larger than the reported FWHM. Adopting a dust temperature of 400 K, we calculate the total infrared luminosity and dust mass to be  $5.9 \times 10^5 L_\odot$  and  $3.9 \times 10^{-4} M_\odot$ , respectively. The derived distance, infrared luminosity and dust mass are similar to the values derived in refs 1 and 3, the main reasons for the differences being the more complete spectral coverage obtained with the additional KAO observations and the adopted dust model. The distance of the dust cloud is similar to that expected for the interaction distance between the winds of the red- and blue-supergiant progenitor<sup>8,9</sup>, suggesting a circumstellar origin for the dust.

As discussed below, the infrared emission could also be due to dust that formed in the supernova ejecta. Roche *et al.*<sup>1</sup>, however, advanced several arguments in favour of an echo interpretation for the emission: 1) scans across the remnant suggest that the emission is resolved, and the projected size of the source implies ejecta velocities of  $\sim 0.37c$ , significantly larger than expected for any dust-forming ejecta; 2) the minimum size of the source cannot be smaller than that of a black body radiating energy at a rate  $L_{\text{IR}} = 5.9 \times 10^5 L_\odot$  at a temperature of 400 K. The derived black-body radius on day 635,  $R_{\text{BB}}$ , is  $1.2 \times 10^{16}$  cm, corresponding to ejecta velocities of  $\sim 2,200$  km  $\text{s}^{-1}$ . If the optical depth  $\tau$  of the ejecta is about unity at 10  $\mu\text{m}$  then for a  $\lambda^{-1}$  emissivity law,  $\tau$  will be  $\geq 20$  at visual band wavelengths. The supernova should therefore be completely obscured in the visual band, contrary to observations (R. M. Catchpole *et al.*, preprint, 1988). One problem with a circumstellar origin for the dust cloud is its special location behind the supernova. Another problem is its implied carbon-rich composition<sup>3</sup>. Silicate particles, with the same radius of 0.1  $\mu\text{m}$ , placed at the distance of the graphite dust cloud will attain a temperature of only 180 K. At this temperature the 18- $\mu\text{m}$  silicate feature is very pronounced (Fig. 1). This feature and the one at 9.7  $\mu\text{m}$  were suggested as spectral signatures of the composition of echoing dust shells around supernovae<sup>10,11</sup>. The 19–23  $\mu\text{m}$  feature in our data could be considered as evidence for the presence of an underlying silicate feature. More detailed calculations show, however, that the feature needs to be narrower and slightly shifted to longer wavelengths to produce a good fit to the 19–23  $\mu\text{m}$  'bump'. We therefore estimate the contribution of silicates to be  $<20\%$  of the total mass of dust giving rise to the infrared emission. This is contrary to the composition of dust expected to form in the oxygen-rich wind<sup>9</sup> of the red-giant progenitor. It may be that the emission is from interstellar dust around the supernova, that somehow was not cleared out of the ambient medium by the blue-supergiant wind.

A different origin for the infrared emission was suggested by Danziger *et al.*<sup>2</sup>, who reported optical evidence for the presence of condensed supernova dust in the expanding ejecta. During August–October 1988, the O I and C I emission lines became asymmetric with their peak emission blueshifted by 500–600 km  $\text{s}^{-1}$ . They attributed this development to extinction by dust that has formed in the metal-rich regions of the expanding ejecta, which may also be responsible for the 8–13  $\mu\text{m}$  infrared emission. (There is a precedent for this phenomenon: line

profiles in SN1979c became asymmetric about eight months after the explosion, with the peak emission blueshifted by  $\sim 700$  km  $\text{s}^{-1}$ , a behaviour consistent with the appearance of dust in the supernova ejecta (R. A. Chevalier and R. F. Kirshner, personal communication). The argument that SN1987A would be completely obscured by the dust<sup>1</sup> can be circumvented by adopting a model for the ejecta in which the dust resides primarily in clumps with  $\tau \approx 1$  and  $\gg 1$  at 10- $\mu\text{m}$  and optical wavelengths, respectively. To comply with the black-body constraints described above, the clumps must subtend a projected area of at least  $\pi R_{\text{BB}}^2$ . To allow for a fraction of the supernova luminosity to escape the clumps need to be embedded within a larger volume of radius  $R$ , approximately given by the requirement that  $N_c(R_c/R)^2 < 1$ , where  $N_c$  and  $R_c$  are the total number of clumps and a typical clump radius, respectively. This gives  $R > R_{\text{BB}}$ . In this simple picture, the observed optical emission arises from the interclump medium with emission from the backside being more highly absorbed by the dust.

The attractive feature of the dust-formation model is that it offers a better explanation for the energy budget of the supernova during the epoch of the infrared emission. We consider the energetics of the supernova on day 613, for which the wavelength coverage is most complete. The total luminosity available on day 613 from the decay of an initial mass 0.078  $M_\odot$  of  $^{56}\text{Co}$  is  $4.87 \times 10^{39}$  erg  $\text{s}^{-1}$  (R. M. Catchpole *et al.*, preprint, 1988). The luminosity observed in the U–M bands is  $1.38(\pm 0.16) \times 10^{39}$  erg  $\text{s}^{-1}$ , the errors reflecting uncertainties in the integration over wavelengths. Various models predicting the X- and  $\gamma$ -ray luminosity  $L_\gamma$  from SN1987A were summarized by Catchpole *et al.* (preprint, 1988). Their predictions for  $L_\gamma$  on day 613 are in the range  $(1.5\text{--}2.5) \times 10^{39}$  erg  $\text{s}^{-1}$ . Of these models, the  $\gamma$ -ray observations on day 433 (ref. 12; N. Gehrels, personal communication) are more consistent with the model of Pinto and Woosley<sup>13</sup>, which predicts  $L_\gamma = 2 \times 10^{39}$  erg  $\text{s}^{-1}$  on day 613. The analysis of the  $\gamma$ -ray data obtained on that day is still in progress (N. Gehrels, personal communication). We will therefore adopt here a value of  $2.0(\pm 0.5) \times 10^{39}$  erg  $\text{s}^{-1}$  for  $L_\gamma$  on day 613, with the error reflecting the range of model predictions. We estimate the infrared luminosity for day 613 to be  $1.7(\pm 0.7) \times 10^{39}$  erg  $\text{s}^{-1}$ . The error in this case reflects the uncertainties in their luminosities for days 580 and 635. Summing up, the total bolometric luminosity from the supernova is  $5.1(\pm 1.4) \times 10^{39}$  erg  $\text{s}^{-1}$ , consistent with the available cobalt decay energy. In the echo model, the infrared and part of the visible light is merely reprocessed light or a reflection from the dust cloud<sup>3</sup>, and should therefore not be included in the bolometric supernova output. The total bolometric luminosity of the supernova would then be  $<3.4(\pm 0.7) \times 10^{39}$  erg  $\text{s}^{-1}$ , which accounts for only  $\sim 70\%$  of the cobalt decay energy.

If the emission originates from newly formed dust then the infrared observations may constitute the 'missing link' between laboratory data of isotope composition anomalies in meteorites and theories on their origin. The dust-formation model does not constrain the composition of the dust in the ejecta, which could be either carbonaceous, silicate or even both, because neither C nor O in the ejecta will be completely locked in CO molecules<sup>14</sup>. The formation of carbonaceous dust in SN1987A, however, could explain the identification by Clayton<sup>15</sup> of the He-rich layers of supernovae as a site for the production of anomalously heavy Xe (formed by the intense neutrino burst). With some of the Xe locked up in carbon grains, this accounts for the presence of these isotopic composition anomalies in meteoritic diamonds<sup>16</sup>.

Continuous infrared observations are extremely important to determine the origin of the infrared emission. In the dust-formation model, the emission will remain spatially unresolved, and its spectral behaviour is determined by the available energy sources in the ejecta at the time of the observations<sup>17</sup>. Consequently, we expect the dust temperature to continuously decrease because of the Co decay and the increasing trans-

parency of the ejecta. With  $\sim 10^{38} \text{ erg s}^{-1}$  as a recently defined upper limit on the luminosity of the underlying pulsar<sup>4</sup>, we do not expect any future rise in dust temperature. In the echo model, the dust temperature decreases due to the combined effects of the Co decay, and the geometry of the dust cloud. Depending on the cloud geometry, we may soon observe spatial as well a spectral evolution in the infrared emission.  $\square$

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## Pulsational magnetic radiation from the neutron star in supernova 1987A

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THE 0.5-millisecond period of pulsar PSR0535-69 in supernova 1987A (ref. 1) may be hard to explain by a conventional rotating neutron-star model, but might more naturally be understood if the neutron star is undergoing radial pulsation<sup>2</sup>. If this is the case, the pulsations will give rise to a time-varying dipole moment, and thus to electromagnetic radiation. I show here that any pulsar heating of the supernova remnant greater than  $\sim 1.5 \times 10^{37} \text{ erg s}^{-1}$  must be due to this pulsational magnetic radiation (PMR). Recent observations<sup>3,4</sup> suggest a pulsar power  $\sim 7 \times 10^{37} \text{ erg s}^{-1}$ . This implies that PMR is indeed heating the supernova nebula, and that the amplitude of the neutron-star surface oscillation after  $\sim 2 \text{ yr}$  is  $\sim 10 B_{12}^{-1} \text{ m}$ , where  $B_{12}$  is the polar magnetic field in units of  $10^{12} \text{ Gauss}$ . The vibrating pulsar model makes several testable predictions: in particular, any PMR component in the light curve will have a characteristic decay time of  $\sim 0.5 \text{ yr}$  (with large uncertainty) which is the result of rotation-induced damping by gravitational radiation. This would be very different from the usual pulsar heating rate, which is approximately flat on timescales of the order of years.

If the 0.5-ms pulses are due to rotation, then bounds on the supernova-remnant energy excess imply a polar field strength  $B \leq 10^9 \text{ G}$  (ref. 1). If, however, the pulses are due to radial oscillations excited at the neutron star's birth, then the requirement that rotation-dependent gravitational radiation does not damp the oscillations too quickly<sup>2</sup> implies  $P_{\text{rot}} > 0.1 \text{ s}$ , and other damping limits described below imply  $B < 10^{13} \text{ G}$ . Comparing these two candidate models with the Crab pulsar ( $P_{\text{Crab}} \approx 20 \text{ ms}$  at birth<sup>5</sup>,  $B_{\text{Crab}} \approx 5 \times 10^{12} \text{ G}$ ), we find that the pulsating model star has  $\geq 5$  times the rotation period and essentially the same magnetic field as the Crab, whereas a 0.5-ms rotator has 1/40th the period and  $\leq 2 \times 10^{-4}$  the field strength. Because the Crab

parameters are typical for observed young pulsars<sup>5</sup>, including those in the Large Magellanic Cloud<sup>6</sup>, here I shall concentrate on the pulsation model.

The energy of the fundamental radial mode of an oscillating neutron star is related to the linear oscillation amplitude  $\delta \equiv \Delta R/R$  by

$$E = 3 \times 10^{53} \delta^2 M_s^2 R_{12}^{-1} \text{ erg} \quad (1)$$

where  $M_s \equiv M/M_\odot$  and  $R_{12} \equiv R/12 \text{ km}$ . This formula is a fit to calculations<sup>7</sup> which employ the Bethe–Johnson equation of state for nuclear matter. The corresponding masses and radii are from ref. 8. The Bethe–Johnson equation of state is in the mid-range of hardness among current candidates<sup>5</sup>. Because of the  $M, R$  scalings, equation (1) is correct to within a factor of roughly two for the other equations of state considered in ref. 7, although it breaks down for neutron stars close to their limiting masses.

Because magnetic flux cannot migrate through the crust on the timescale of a pulsation period, the magnetic-field variations are confined to the surface and so vary as  $R^{-2}$  during pulsations. This leads to a time-varying dipole moment,

$$|\mathbf{M}| = \frac{BR^3}{2} \propto R(t) \quad (2)$$

where  $B$  is the field strength at the magnetic pole. The resulting PMR has power

$$\dot{E}_{\text{PMR}} = \frac{B^2 R^6 \omega_{\text{pls}}^4 \delta^2}{12c^3} \quad (3)$$

where  $\omega_{\text{pls}}$  is the pulsation angular frequency  $2\pi/P_{\text{pls}}$ . This mode of neutron-star emission has been independently suggested by a number of authors<sup>9–11</sup>.

A comparison of equations (1) and (3) shows that PMR damps the pulsation energy on an folding timescale (defined as  $-E/E$ )

$$\tau_{\text{PMR}} \approx 40 B_{12}^{-2} R_{12}^{-7} M_s^2 \text{ yr} \quad (4)$$

The condition that PMR does not damp out the oscillations before the time at which they were observed implies

$$B \leq 10^{13} R_{12}^{-7/2} M_s \text{ G} \quad (5)$$

Together with the gravitational-wave damping limit  $P_{\text{rot}} > 0.1 R_{12}^{1/2} M_s^{1/4} \text{ s}$  mentioned above<sup>2</sup>, this implies that the rotational magnetic-dipole radiation, RMR (that is, the component of emitted power peaked near  $\omega_{\text{rot}} \equiv 2\pi/P_{\text{rot}}$ ), is

$$\dot{E}_{\text{RMR}} < 1.4 \times 10^{37} R_{12}^{-3} M_s \text{ erg s}^{-1} \quad (6)$$

A more rigorous argument confirms this result<sup>12</sup>.

There is tentative evidence that a central pulsar is energizing the SN1987A remnant at a rate (on day  $\sim 750$ ) just slightly below  $\sim 10^{38} \text{ erg s}^{-1}$ . On 1989 March 6 it was announced<sup>3</sup> that the bolometric light curve exhibits a steady component of  $7 \times 10^{37} \text{ erg s}^{-1}$ . When this component is subtracted from the data, a constant exponential decline is found from day 520 to 730 (ref. 3). Furthermore, soft X-rays (6–10 keV) observed by the Ginga satellite have been interpreted as synchrotron emission from a central non-thermal nebula powered at the  $\sim 10^{38}$  level<sup>4,13</sup>. If remnant heating in this range is verified by future studies, then equation (6) implies that the low-frequency RMR is not adequate to power the nebula and one must conclude that kHz-frequency pulsational magnetic radiation is responsible for heating the SN1987A nebula.

In a PMR-heated supernova, the amplitude of the radial pulsations can be estimated using equation (3):

$$\delta = 6 \times 10^{-4} B_{12}^{-1} R_{12}^{-3} L_7^{1/2} \quad (7)$$

where  $L_7$  is the pulsar power in units of  $7 \times 10^{37} \text{ erg s}^{-1}$ . For homologous motion (which is a reasonably accurate assumption<sup>7</sup>) and  $L_7 = 1$  this corresponds to a degenerate surface oscillation of  $\Delta R \approx 14 B_{12}^{-1} R_{12}^{-2} \text{ m}$ , peak to peak. Adopting the upper



limit on  $B$  from equation (5), which agrees with empirical properties of the pulsar population<sup>5</sup>, I obtain a lower limit on the amplitude  $\delta > 6 \times 10^{-5} L_1^{1/2}$  or (roughly)  $\Delta R > 1.4$  m. Of course, plasma effects close to the pulsar (for which there is evidence in SN1987A, as mentioned above<sup>4,13</sup>) complicate the idealized emission mechanism described here and might change these results by factors of the order of unity<sup>5</sup>.

Although PMR heats the nebula, rotation-induced gravitational radiation is the dominant pulsation-damping mechanism (unless either  $P_{\text{rot}} > 0.3 B_{12}^{-1/2}$  s or neutrino damping is enhanced by some exotic interior composition<sup>2</sup>) with a timescale

$$\tau_{\text{GR}} \approx 0.5 \left( \frac{P_{\text{rot}}}{0.1 \text{ s}} \right)^4 \text{ yr} \quad (8)$$

The only detailed, published calculation<sup>14</sup> of  $\tau_{\text{GR}}$  is based on uniformly rotating constant-density model stars undergoing homologous radial oscillations. (Reference 15 contains an earlier estimate.) Real neutron stars are centrally condensed by a factor of roughly four or more, depending on the uncertain equation of state at supranuclear densities<sup>8</sup> and a crude estimate (L. Jin, personal communication) shows that this decreases the efficiency of gravitational-wave emission by a factor of roughly two from the value in ref. 14. This factor of two has been included in the above estimate.

As an illustrative calculation, I express the theoretical uncertainty in  $\tau_{\text{GR}}$  in terms of a parameter  $\eta$ . The initial amplitude of oscillations of the neutron star becomes

$$\delta_0 \approx \delta \exp \left[ 2\eta^{-1} \left( \frac{P_{\text{rot}}}{0.1 \text{ s}} \right)^{-4} \right] \quad (9)$$

where  $\delta$  is the present amplitude ( $\sim$ day 700). Using  $P_{\text{rot}} = 0.1$  s and  $\eta = 1$ , the initial amplitude was  $\delta_0 \approx 0.01$  ( $\delta/10^{-3}$ ). This implies that the nascent neutron star had a radial oscillation energy  $E_0 \approx 3 \times 10^{49} \delta_3^2$  ergs, where  $\delta_3 \equiv \delta/10^{-3}$ . This energy was presumably left over after the star's core bounce was damped by driving the supernova shock; the fundamental radial mode might be excited with comparable strength in all nascent neutron stars. Most of the pulsational energy was lost to gravitational radiation. The total energy imparted to the supernova nebula by PMR since birth of the neutron star is

$$E_{\text{neb}} = \frac{E_0 \tau_{\text{GR}}}{\tau_{\text{PMR}}} \approx 10^{-2} E_0 \left( \frac{P_{\text{rot}}}{0.1 \text{ s}} \right)^4 B_{12}^{-2} \quad (10)$$

In the case considered above,  $E_{\text{neb}} \approx 3 \times 10^{47} \delta_3^2$  erg. The greatest share of this was emitted in the first  $\tau_{\text{GR}}$  and increased the supernova explosion energy by only  $\sim 3$  parts in  $10^4$  for  $\delta_3 = 1$ . Note that  $\tau_{\text{GR}}$  is  $\sim 3\eta$  times the present decay time of the radioactive  $^{56}\text{Co}$  component of the light curve (which has been gradually diminishing because of increasing transparency to  $\gamma$ -rays<sup>3,16</sup>) so it is understandable that the PMR component of the light curve became evident only after  $\sim 2$  yr.

Attempts by the original observers<sup>1</sup> and others<sup>17</sup> to reobserve the 0.5-ms pulses have failed. This fact, along with the change in the pulse luminosity during the original observing run, have led to speculation that variable dust obscuration has been present<sup>1</sup>. The apparent blueshifts of emission lines give evidence for recent dust formation in the supernova nebula<sup>18</sup>. Furthermore, observations of the SN1987A infrared excess made with the Kuiper Airborne Observatory suggest that clumpy dust has formed<sup>19</sup>. Calculations show that temperatures could reach the dust condensation temperature in the metal-rich mantle as early as day  $\sim 600$  (ref. 20).

If the neutron star is truly obscured, it still might be possible to corroborate the pulsation model because it makes very distinctive predictions for the supernova light curve. (Of course, the effect of dust on the light curve itself must be taken into account.) After radioactive heating drops below  $\dot{E}_{\text{PMR}}$  at a crossover time  $t_1$ , the bolometric luminosity  $L$  enters a phase of more gentle

exponential decline:

$$L(t) = L_1 \exp \left( -\frac{t-t_1}{\tau_1} \right) \quad (11)$$

where

$$\tau_1 \equiv (\tau_{\text{GR}}^{-1} + \tau_{\text{PMR}}^{-1} + \tau_{\nu}^{-1})^{-1} \quad (12)$$

In this equation  $\tau_{\nu}$  is the neutrino damping time, which is  $\sim 10^2$  yr unless some exotic enhancement occurs<sup>2,21</sup> so this term is probably not important. Furthermore, if  $P_{\text{rot}} < 0.3 B_{12}^{-1/2}$  s, as seems likely, then  $\tau_1 \approx \tau_{\text{GR}}$ .

The light curve should therefore show a  $\tau_{\text{GR}} \sim 200$ -day slope to the log  $L$  (pulsar) component. This is very different from the flat (on a timescale of years) contribution of rotation-driven pulsar heating.

This circumstance underscores the need for diligent monitoring of the light curve during the next two years or so. Of course, the precise value of  $\tau_{\text{GR}}$  is difficult to predict because of its steep dependence on  $P_{\text{rot}}$  and present theoretical uncertainties (compare equation (8)).

The luminosity phase of equation (11) persists until  $L(t)$  reaches a value at time  $t_r$

$$L_2 \approx 10^{35} B_{12}^2 \left( \frac{P_{\text{rot}}}{0.1 \text{ s}} \right)^{-4} R_{12}^6 \text{ erg s}^{-1} \quad (13)$$

at which time the low-frequency rotational luminosity becomes important and the light curve asymptotically reaches

$$L(t) = \frac{L_2 \tau_2^2}{[\tau_2 + (t-t_2)]^2} \quad (14)$$

where  $\tau_2$  is the energy decay rate due to spindown at time  $t_2$ :

$$\tau_2 \equiv \frac{1}{2} I \omega_2^2 L_2^{-1} = 10^5 B_{12}^{-2} I_{45} \left( \frac{P_{\text{rot}}}{0.1 \text{ s}} \right)^2 \text{ yr} \quad (15)$$

where  $I_{45}$  is the moment of inertia in units of  $10^{45}$  g cm<sup>2</sup> and  $\omega_2$  is the rotational angular frequency at time  $t_2$ . The decline in this final phase is undetectably small, although the associated  $\dot{P} = P_{\text{rot}}/\tau_2 \approx 10^{-14}$  could be detectable if the pulsar is detected at radio frequencies.

Measuring the decay time of the pulsar contribution to the light curve at  $t < t_2$  will determine  $P_{\text{rot}}$ . If the levelling off at  $L_2$  is detected (which may require waiting until the contribution from long-lived isotopes like  $^{57}\text{Co}$  decreases as a result of decreasing  $\gamma$ -ray absorption<sup>13</sup>) this will determine  $B$ . In this way one might deduce the amplitude of the initial oscillations through equations (7) and (9) without seeing the central star again. If a radio pulsar is found,  $P_{\text{rot}}$  will be directly measured and  $B$  determined from  $\dot{P}$ , thus the pulsation model could conceivably be confirmed without any second observation of the 0.5-ms pulses.  $\square$

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# The acceleration of pulsars—a new test

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If pulsars are accelerated by the emission of asymmetric magnetic dipole<sup>1</sup> or neutrino<sup>2</sup> radiation, the spatial velocity acquired should lie along its axis of rotation. A simple test<sup>3</sup> of such models is that the position angle on the sky of the proper motion  $\varphi_v$  and the polarization plane at the peak of the pulse profile  $\varphi_p$  should be either parallel or perpendicular. This hypothesis does not appear to be borne out by the available observations<sup>4</sup>. We have proposed<sup>5</sup> a more direct 'scalar' test of the acceleration idea: the tangential velocity  $V_t$  of a pulsar (that is, the projection of its actual spatial velocity) should be proportional to the sine of the angle  $\zeta$  between the line of sight and the rotation axis.

Table 1 presents data for 29 pulsars with well-known tangential velocities<sup>6–10</sup> and known distances<sup>8,11</sup>. We used estimates of the angles  $\beta$  between the magnetic and rotational axes as well as the values  $\zeta = \beta + |\zeta - \beta|$  and  $|\zeta - \beta|$  for 21 pulsars from ref. 12. Average values of  $\zeta$  were used for all cases except that of PSR 0833–45 for which the smaller value was used. The statistical method for the determination of these angles<sup>12</sup> uses the maximum rate of change of the internal position angle of the polarization plane and the value of the cone semiangle of the pulsar radiation, as in ref. 13. However, the angles  $\theta$  for the 400-MHz frequency in ref. 12 are 1.42 times larger than in ref. 13 and the values of the angles  $\beta$  and  $\zeta$  are therefore systematically greater.

Using reduction formulae we obtained estimates of  $\zeta$  and  $|\zeta - \beta|$  for PSR0329+54, 0736+40, 1541+09 and 2020+28 from the data in ref. 13. For PSR0355+54 and 1604–00 we obtained estimates of  $\zeta$  in the same manner as in ref. 13.

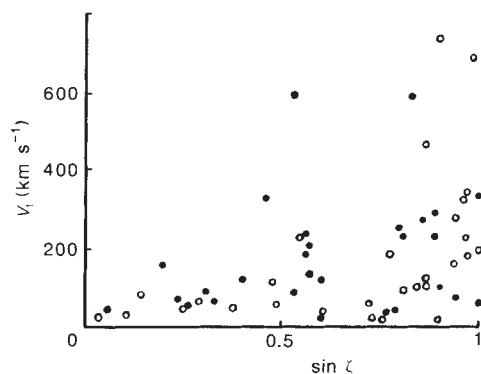


FIG. 1 Tangential velocities of pulsars as a function of  $\sin \zeta$ . Open circles refer to pulsars in Table 1. The filled circles refer to 28 other pulsars for which  $\zeta$  and  $V_s$ , the velocity of the interstellar-medium producing scintillation of the pulsars, are known<sup>11</sup>. The values of  $V_s$  are, on average, smaller than  $V_t$  by a factor of 2.1.

We used position angles  $\varphi_p$  of the polarization planes at the centres of the pulse profiles of radio emission from refs 14–16, where possible, we used recent estimates of the Faraday-rotation measure<sup>17</sup>. Where the angle of the plane of polarization inside the pulse profile changed abruptly, the angle  $\varepsilon = |\varphi_p - \varphi_v|$  increased by 90° as reported in ref. 14 except for the case of PSR0355+54; these cases are marked by asterisks. The last column of the table gives the estimates of the spatial velocities,  $V = V_t / \sin \zeta$ .

Figure 1 shows the correlation of  $V_t$  and  $\sin \zeta$ . The model of the acceleration of the pulsar along the rotation axis and the model of its radiation through the polar cap<sup>18</sup>, heretofore unrelated, are mutually justified.

The mean spatial velocity of the pulsars referred to in Table 1 is  $257 \pm 37 \text{ km s}^{-1}$ , for the additional 28 pulsars  $\bar{V}$  is  $308 \pm 47 \text{ km s}^{-1}$ , and for the whole set  $\bar{V}$  is  $282 \pm 38 \text{ km s}^{-1}$ . The

TABLE 1 Kinematic data of pulsars

| PSR     | Distance (kpc) | $V_t$ (km s <sup>-1</sup> ) | $ \zeta $ (deg.) | $ \beta - \zeta $ (deg.) | $\varphi_p$ (deg.) | $\varphi_v$ (deg.) | $\varepsilon$ (deg.) | $V$ (km s <sup>-1</sup> ) |
|---------|----------------|-----------------------------|------------------|--------------------------|--------------------|--------------------|----------------------|---------------------------|
| 0301+19 | 0.56           | 101±14                      | 57               | 3.4                      | 23±2               | -9±11              | 32±11                | 121±16                    |
| 0329+54 | 2.30           | 225±25                      | 33               | 2.9                      | 30±13              | -56±2              | 86±13                | 412±46                    |
| 0355+54 | 1.64           | 66±31                       | 17               | 1.5                      | 138±2              | 41±27              | 83±27                | 226±106                   |
| 0531+21 | 2.00           | 157±41                      | 69               | 16.0                     | 140±1              | -64±6              | 24±6                 | 168±44                    |
| 0611+22 | 3.07           | 131±57                      | 60               | 9.6                      | 16±1               | 0±43               | 16±43                | 151±66                    |
| 0736-40 | 2.00           | 684±80                      | 80               | 7.3                      | —                  | -50±8              | —                    | 694±81                    |
| 0809+74 | 0.18           | 43±6                        | 14               | 3.8                      | 152±5              | 163±8              | 79±9*                | 178±25                    |
| 0823+26 | 0.36           | 193±19                      | 88               | 3.1                      | 10±3               | -33±1              | 83±3                 | 193±19                    |
| 0833-45 | 0.50           | 36±13                       | 37               | 14.5                     | 63±2               | -126±16            | 9±15                 | 60±22                     |
| 0834+06 | 0.43           | 105±16                      | 60               | 4.3                      | 123±9              | 2±6                | 59±11                | 121±18                    |
| 0943+10 | 0.56           | 115±59                      | 28               | 6.8                      | —                  | -119±17            | —                    | 245±126                   |
| 0950+08 | 0.13           | 25±3                        | 6                | 6.8                      | 172±6              | 27±6               | 35±8                 | 239±29                    |
| 1133+16 | 0.16           | 278±28                      | 70               | 6.0                      | 117±7              | -12±1              | 51±7                 | 296±30                    |
| 1237+25 | 0.33           | 178±18                      | 76               | 2.3                      | 76±4               | -68±1              | 36±4                 | 183±18                    |
| 1508+55 | 0.73           | 346±36                      | 76               | 5.8                      | 8±4                | -133±2             | 39±4                 | 357±46                    |
| 1541+09 | 1.34           | 81±31                       | 8                | 1.0                      | 45±12              | -75±14             | 60±18                | 583±223                   |
| 1604-00 | 0.36           | 14±15                       | 63               | 14.4                     | 175±10             | -174±22            | 11±24                | 16±19                     |
| 1642-03 | 0.16           | 20±8                        | 47               | 0.8                      | 65±15              | 168±12             | 77±19                | 87±11                     |
| 1818-04 | 1.50           | 187±25                      | 51               | 3.9                      | 79±2               | 6±7                | 73±2                 | 241±32                    |
| 1929+10 | 0.11           | 55±6                        | 29               | 8.1                      | 65±5               | 64±3               | 1±6                  | 113±6                     |
| 1933+16 | 6.00           | 733±130                     | 64               | 12.5                     | 167±9              | 175±8              | 8±12                 | 816±145                   |
| 1944+17 | 0.47           | 20±9                        | 2                | 1.7                      | 101±9              | -6±32              | 73±2                 | 571±257                   |
| 1952+29 | 0.21           | 44±11                       | 22               | 5.8                      | —                  | 145±20             | —                    | 117±29                    |
| 2016+28 | 1.30           | 15±13                       | 49               | 6.4                      | 94±2               | 57±24              | 53±24*               | 20±17                     |
| 2020+28 | 1.30           | 97±15                       | 54               | 3.0                      | 179±10             | 35±10              | 36±14                | 120±18                    |
| 2021+51 | 0.70           | 59±13                       | 46               | 7.6                      | 29±2               | 19±13              | 10±13                | 82±18                     |
| 2045-16 | 0.39           | 465±86                      | 60               | 1.7                      | 91±21              | 7±3                | 84±21                | 537±100                   |
| 2217+47 | 1.46           | 225±47                      | 76               | 6.3                      | 81±6               | 22±14              | 59±15                | 232±48                    |
| 2303+30 | 1.91           | 321±63                      | 73               | 7.0                      | —                  | 159±13             | —                    | 336±66                    |



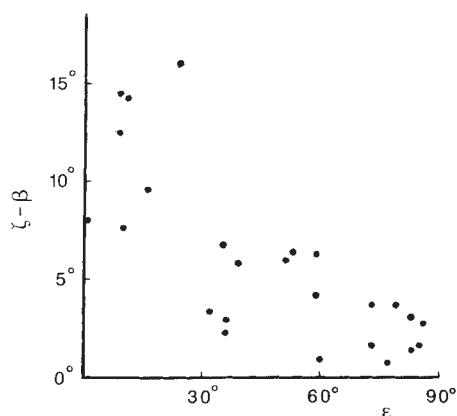


FIG. 2  $|\zeta - \beta|$  as a function of  $\epsilon$ .

exclusion, in both samples, of three pulsars having small  $\zeta$ -values gives  $\bar{V} = 233 \pm 38 \text{ km s}^{-1}$  for 26 pulsars from Table 1,  $\bar{V} = 246 \pm 29 \text{ km s}^{-1}$  for 25 pulsars from additional sample and  $\bar{V} = 224 \pm 29 \text{ km s}^{-1}$  for the whole set. Spatial velocities  $> 500 \text{ km s}^{-1}$  are obtained for five of the pulsars mentioned in the table and for five of the pulsars from the additional set. In both of these sets two pulsars have small  $\zeta$ -values of less than  $11^\circ$  and therefore their values of  $V$  are unreliable. Three pulsars in the additional set have anomalously large values of the interstellar-medium velocity  $V_s$  and the distances of PSR0736-40

and 1933+16 have probably been overestimated. Therefore the only pulsar for which there are no suspicions about the high velocity  $V$  is PSR2045-16 and so we believe that it would be premature to postulate the existence of a secondary maximum in the velocity distribution above  $500 \text{ km s}^{-1}$ .

The data in the table indicate a uniform  $\epsilon$ -distribution, which is in agreement with a previous study<sup>4</sup>. The postulate<sup>3</sup> that the values of  $\epsilon$  may be only  $0^\circ$  and  $90^\circ$  is incorrect. Figure 2 shows the relationship between  $\epsilon$  and  $|\zeta - \beta|$ , which may be attributed to the change in  $\zeta$  for different intersections of the pulsar radiation cone with the line of sight. Therefore, the value of  $\epsilon$  changes monotonically from  $0^\circ$  for  $|\zeta - \beta| \approx \theta$ , to  $90^\circ$  for  $|\zeta - \beta| \approx 0^\circ$ . □

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## Submarine venting of phase-separated hydrothermal fluids at Axial Volcano, Juan de Fuca Ridge

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SINCE the discovery of high-temperature venting on the East Pacific Rise in 1979<sup>1</sup>, it has been expected<sup>2,3</sup>, because of the physical properties of sea water at pressures and temperatures encountered during submarine hydrothermal circulation<sup>4,5</sup>, that phase-separated fluids would discharge from ridgecrest vents. Although this notion is supported by the reported large deviations in vent-fluid chlorinity relative to that of sea water ( $-40\%$ – $+200\%$ )<sup>6,7</sup>, by observations of venting at  $P$ – $T$  conditions clearly within the two-phase region (220 bar and  $420^\circ\text{C}$ )<sup>8</sup> and by fluid-inclusion data<sup>9,10</sup>, unequivocal identification<sup>11</sup> of phase-separated venting fluids has remained elusive. Here we report observations of chloride- and metal-depleted, gas-enriched fluids from a shallow vent field on the Juan de Fuca Ridge which confirm the expectation that phase-separated effluents are delivered to the deep ocean from some sea-floor venting systems.

The ASHES (Axial Seamount Hydrothermal Emissions Study) vent field lies at a depth of 1,542 m within the caldera

of Axial Volcano on the Juan de Fuca Ridge (Fig. 1) and is the surface expression of the shallowest active high-temperature sea-floor venting system known<sup>12</sup>. Within a 60-m-diameter area located at the northern end of ASHES, four sulphide edifices (Inferno, Hell, Hillock and Mushroom, Fig. 1) rise 1–4 m above bare lobate basalt and vent clear, brown and black fluids at temperatures reaching  $328^\circ\text{C}$ . Clear, high-temperature fluids also discharge from two unusual vent structures located within this region: Virgin Mound ( $T_{\text{max}} = 299^\circ\text{C}$ ), a  $< 0.7$ -m tall, white anhydrite chimney that rebuilds  $< 24$  h after demolition, and Crack Vent ( $T_{\text{max}} = 219^\circ\text{C}$ ), a several metre long, 7- to 10-cm wide, anhydrite-filled fracture in sheet-flow basalt. Low-temperature ( $T_{\text{max}} = 55^\circ\text{C}$ ) fluids issue from smaller cracks located above the vent field.

Vent-fluid samples were collected at the ASHES site in July 1986 using the research submersible *Pisces IV*<sup>12</sup>, and in September 1987 and August 1988 with DSV *Alvin*. The results for selected vent-fluid properties are shown in Fig. 2 and the hydrothermal endmember compositions, estimated by extrapolation of the entire data set to zero Mg concentration<sup>13</sup>, are given in Table 1. The low Mg values observed and the reproducible trends of the ASHES data throughout the two-year sampling period (Fig. 2) support the use of the above method for estimating endmembers.

Because  $\text{Cl}^-$  is the major anion in vent fluids and because chlorinity serves as an approximate measure of the total dissolved salts, we have arranged the vent-fluid data into three groups based on endmember Cl concentrations relative to the ambient seawater value of  $539 \text{ mmol kg}^{-1}$  (Fig. 2, Table 1). Inferno is the only vent sampled with a Cl concentration significantly greater than that of sea water,  $625 \text{ mmol kg}^{-1}$ ; data from Inferno is referred to as Cl-enriched. Hell, Hillock, and Mushroom vent fluids have endmember Cl concentrations in the range 480–540  $\text{mmol kg}^{-1}$  (composite value  $515 \text{ mmol kg}^{-1}$ ) and are here termed Cl-normal. Cl-depleted fluids were collected from Virgin Mound, Crack and low-temperature vents and for these the endmember Cl concentrations are in the range 176–258  $\text{mmol kg}^{-1}$  (composite value  $188 \text{ mmol kg}^{-1}$ ). The Cl-depleted fluids are also notably depleted in metals and enriched

in gases. The observation of such large variations in chlorinity within a single vent field, with different vent types sometimes located within only a few metres of one another, is unprecedented.

The abnormally low Cl and metal and high gas concentrations of the Cl-depleted fluids are the most remarkable attributes of the ASHES data. The Cl-endmember concentration is 35% of the Cl concentration of sea water and is by far the lowest reported value for ridgecrest fluids. Rock hydration and variable precipitation/dissolution of an unidentified chloride-bearing mineral, which have been proposed to explain Cl concentrations differing from that of sea water at other ridgecrest systems, are not adequate to explain the magnitude of the ASHES Cl depletion<sup>6,7,13-15</sup>. Endmember values of Fe, Zn and Mn are only 1%, 2% and 16% of the respective Cl-normal values and are much lower than concentrations reported for other bare basalt high-temperature venting sites<sup>6,7,14,16</sup>. The higher pH, cooler temperature and lower Cl concentration of the vapour-enriched fluids probably all contribute to the low metal loadings<sup>6,17</sup>. The total condensable-gas concentration of the Cl-enriched fluid (50 mmol kg<sup>-1</sup>, assumed to be CO<sub>2</sub> because H<sub>2</sub>S was removed before analysis) is similar to that predicted from an earlier measurement of warm spring discharge at the Canadian-American Seamount (CASM) site<sup>18</sup> (Fig. 1) and is an order of magnitude higher than values reported for fluids from other unsedimented ridgecrests<sup>6,19</sup>. Even more striking and significant is that the gas concentration (285 mmol kg<sup>-1</sup>) of the Cl-depleted endmember is almost six times higher than that of the Cl-enriched endmember.

We believe that the unusual characteristics of the Cl-depleted endmember can be best explained by phase separation, phase segregation<sup>20</sup> (that is, unmixing, to various degrees, of the vapour

and liquid phases) and, finally, addition of a small amount of cold sea water to the vapour-enriched mixture. We know of no other mechanism capable of producing the large deficits of Cl, Br and Na in the Cl-depleted fluids. The fact that the concentrations of Li and K, species which are conservative during sea-water mixing, from all of the high-temperature vents at ASHES can be described (after removal of the seawater component of the mixtures) by a single mixing line having a freshwater-like fluid as its origin (Fig. 3), is strong support for the hypothesis that phase separation and segregation are important processes that control venting chemistry at this site.

Maximum venting temperatures of 295 °C, 298 °C and 299 °C were measured at Virgin Mound during 1986, 1987 and 1988, respectively. For Inferno vent, the corresponding maximum temperatures were 328 °C, 326 °C and 319 °C. Although the gas concentration at Inferno is sufficient to lower the sea-floor boiling temperature by 3–5 °C (ref. 21), all of the measured temperatures are at least 20 °C below the sea-floor boiling temperature (348 °C (ref. 22)), indicating that some combination of conductive heat transfer to host rock and/or mixing with cold sea water has taken place. As heat loss accompanies boiling and because pressures are greater in the subsurface, initial boiling temperatures must have been significantly greater than 350 °C.

The relative gas concentrations of the Cl-enriched and Cl-depleted endmembers provide critical support for the phase-separation hypothesis<sup>6,11</sup>. By extrapolating gas solubility data for sub-critically boiling salt solutions beyond 350 °C (ref. 17), a fully segregated vapour formed by boiling sea water at, for example, 370 °C should be ten times more concentrated in CO<sub>2</sub> and five times more concentrated in H<sub>2</sub>S, relative to the residual liquid phase. Gas partitioning to the vapour phase would be reduced for boiling at higher temperatures. Although uncertainty

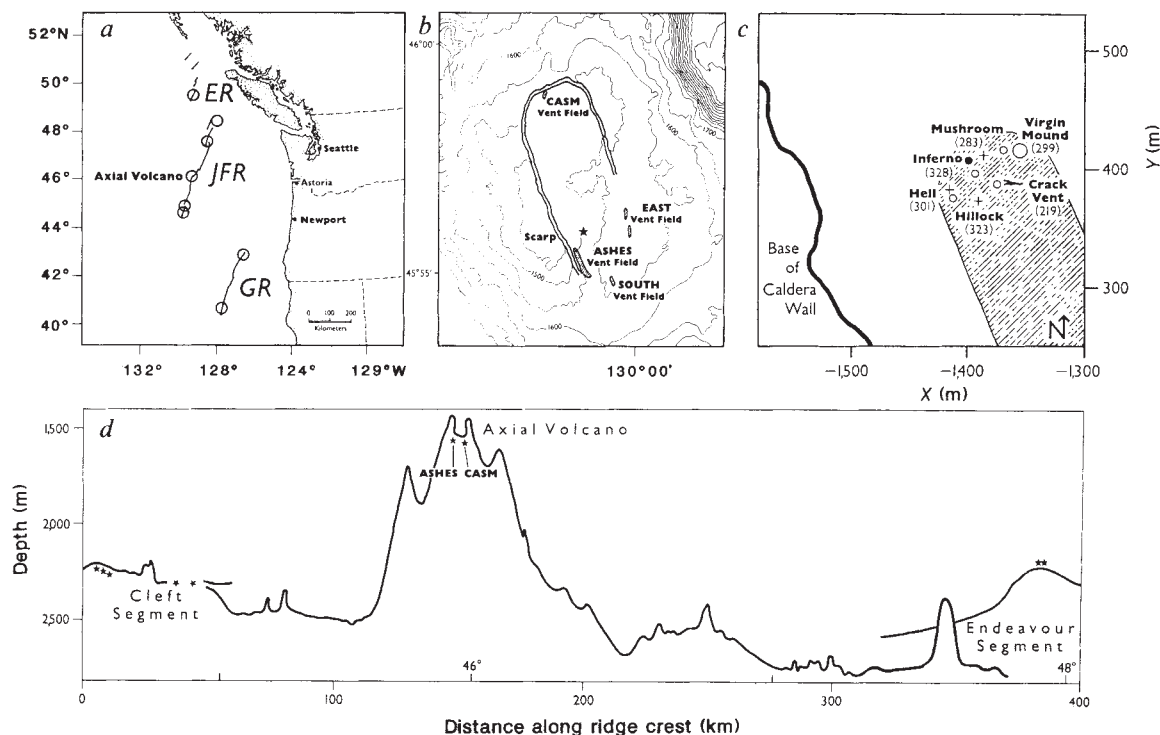


FIG. 1 *a*, Location map of Axial Volcano on Juan de Fuca Ridge (JFR), north-east Pacific Ocean. Explorer Ridge (ER) and Gorda Ridge (GR) also shown. Circles mark known venting sites. *b*, Position of ASHES vent field within caldera of Axial Volcano: ★ tied to high-temperature venting region that is confined to northern end of vent field. CASM, East and South vent fields support low-temperature ( $T \leq 100$  °C) venting<sup>18,28</sup>. Metal-sulphide- and barium-enriched chimneys, evidence for past high-temperature discharge, have been recovered from CASM and East sites. Contour interval of bathymetry, 50 m. *c*, Detail map of high-temperature venting domain within ASHES vent field sampled in 1986, 1987 and 1988. High-temperature

vents: ● and +, 1–4 m high sulphide edifices with multiple orifices; ○ and —, <0.7-m-high anhydrite chimney and several metre-long, 7–10 cm wide anhydrite-filled cracks. Maximum observed venting temperatures given in parentheses (°C). Low-temperature ( $T \leq 55$  °C) vent sampling sites, ○. Vent structures sit on unsedimented lobate and sheet-flow basalt at depth of  $1,542 \pm 1$  m. Coordinates are relative to acoustic navigation net ( $x = 1375$ ,  $y = +400$  equivalent to GPS position:  $45^{\circ}56.0'N$ ,  $130^{\circ}00.9'W$ ). *d*, Juan de Fuca ridgecrest bathymetry showing relatively shallow depth of ASHES vent field. Stars mark locations of known vent fields.

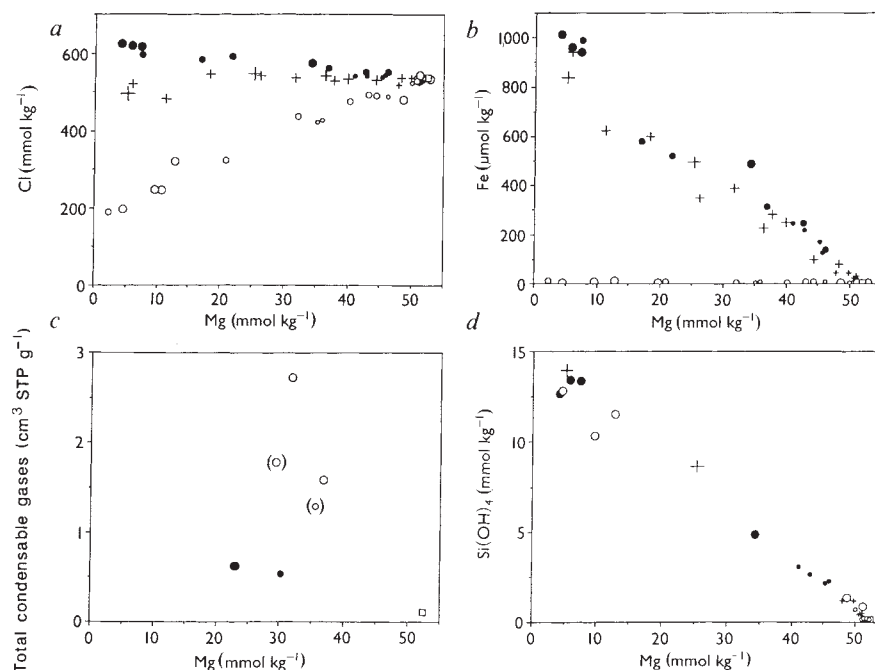


TABLE 1 Endmember values for ASHES vent fluids and regional sea water

|                                                       | Cl-enriched      | Cl-normal | Cl-depleted      | Sea water |
|-------------------------------------------------------|------------------|-----------|------------------|-----------|
| Temperature (°C)                                      | 149–328          | 136–323   | 5–299            | 2.42      |
| pH (NBS, 25 °C)                                       | 3.5              | 3.5       | 4.4              | 7.47      |
| Alkalinity ( $\mu\text{eq kg}^{-1}$ )                 | –453             | –519      | 580              | 2,354     |
| Condensable gases ( $\text{cm}^3 \text{STP g}^{-1}$ ) | 1.1              | ND        | 6.3              | 0.05      |
| $\text{H}_2\text{S}$ ( $\text{mmol kg}^{-1}$ )        | 7.0 (7.5)        | 8.1       | (19.5)           | 0         |
| $\text{Si(OH)}_4$ ( $\text{mmol kg}^{-1}$ )           | 14.7             | 15.8      | 13.8             | 0.169     |
| Mg ( $\text{mmol kg}^{-1}$ )                          | 0                | 0         | 0                | 52.4      |
| Cl ( " " )                                            | 625              | 515       | 188              | 539       |
| Na ( " " )                                            | 500              | 415       | 159              | 462       |
| Ca ( " " )                                            | 46.8             | 37.3      | 10.2             | 10.3      |
| K ( " " )                                             | 27.5             | 22.0      | 7.6              | 9.8       |
| Br ( " " )                                            | 0.95             | 0.76      | 0.24             | 0.83      |
| Li ( " " )                                            | 0.637            | 0.512     | 0.204            | 0.026     |
| Mn ( $\mu\text{mol kg}^{-1}$ )                        | 1,133            | 1,016     | 162              | 0.001     |
| Fe ( " " )                                            | 1,071            | 925       | 9                | 0.009     |
| Cu ( " " )                                            | 12               | 10        | 0.7              | 0.004     |
| Zn ( " " )                                            | 114              | 115       | 2.3              | 0.009     |
| Pb ( " " )                                            | 0.34             | 0.30      | 0.10             | 0.00001   |
| P ( " " )                                             | 0.09             | 0.30      | 0.31             | 3.07      |
| Ge ( " " )                                            | 0.145            | 0.148     | 0.126            | 0.000122  |
| B ( " " )                                             |                  |           |                  | 421       |
| $\delta^{18}\text{O}$ (‰)                             | 565<br>+0.8–+1.1 |           | 503<br>+0.7–+0.9 | –0.2      |

Water samples were collected in 755-ml titanium-piston syringes<sup>14</sup>; total condensable-gas samples in 150-ml titanium gas-tight bottles (supplied by J. Lupton). In 1987 and 1988 titanium sample reservoirs were coupled to an insulated manifold sampling system equipped with a platinum-resistance thermometer (precision:  $\pm 1^\circ\text{C}$ ) at the intake nozzle; range of collection temperatures given. pH (precision: 0.3%) and alkalinity (0.2%) determined potentiometrically. Total condensable gases (3%) determined manometrically;  $\text{H}_2\text{S}$  removed by precipitation as  $\text{HgS}$  before determination.  $\text{H}_2\text{S}$  (5%),  $\text{Si(OH)}_4$  (1%), and P (2%) determined by flow-injection visible spectrophotometry; Mg (0.5%) and Ca (0.5%) by EDTA–EGTA titration; C by potentiometric titration (0.2%); Br (5%) by visible spectrophotometry; K (4%), Li (2%), Mn (2%), Fe (7%) and Zn (2%) determined by flame atomic absorption or emission spectrophotometry; Cu (2%), Pb (5%), and, at low concentrations, Mn (5%), Fe (5%) and Zn (5%), determined by graphite-furnace atomic absorption spectrophotometry; B (3%) determined by inductively-coupled-plasma emission spectrometry; Na estimated by charge balance. Analysis of Ge by R. A. Mortlock  $\delta^{18}\text{O}$  relative to standard mean ocean water, contributed by W. C. Shanks III and J. K. Böhlke.  $\text{H}_2\text{S}$  endmembers in parentheses were estimated from weights of  $\text{HgS}$  in gas-tight samples. An  $\text{H}_2\text{S}$  endmember for Cl-depleted fluids collected in Ti-syringe samplers is not reported because, on several occasions, these gas-enriched samples were observed to de-gas (bubble) during ascent from the sea floor. Cl-enriched and Cl-normal data combined for estimation of B and  $\delta^{18}\text{O}$  endmembers. ND, not determined.

FIG. 2 Values of *a*, Cl ( $\text{mmol kg}^{-1}$ ), *b*, Fe ( $\mu\text{mol kg}^{-1}$ ), *c*, total condensable gases ( $\text{cm}^3 \text{STP g}^{-1}$ , assumed here to be  $\text{CO}_2$ ; see text) and *d*,  $\text{Si(OH)}_4$  ( $\text{mmol kg}^{-1}$ ) plotted against Mg ( $\text{mmol kg}^{-1}$ ) for ASHES vent fluids. Data symbols: ●, Inferno; +, Mushroom, Hell and Hillock; ○, Virgin Mound, Crack Vent, miscellaneous warm vents; □, sea water. Symbol sizes increase with each year group; 1986 smallest, 1988 largest. Total condensable-gas values in parentheses are from Crack Vent (higher value) and were not considered in estimation of endmember. Missing data for  $\text{Si(OH)}_4$  is the result of sample-storage artefacts. Because pure hydrothermal fluids are assumed to be totally depleted in Mg (ref. 14), hydrothermal endmember values are estimated by extrapolation of linear-regression lines for selected data trends to corresponding zero Mg concentrations.

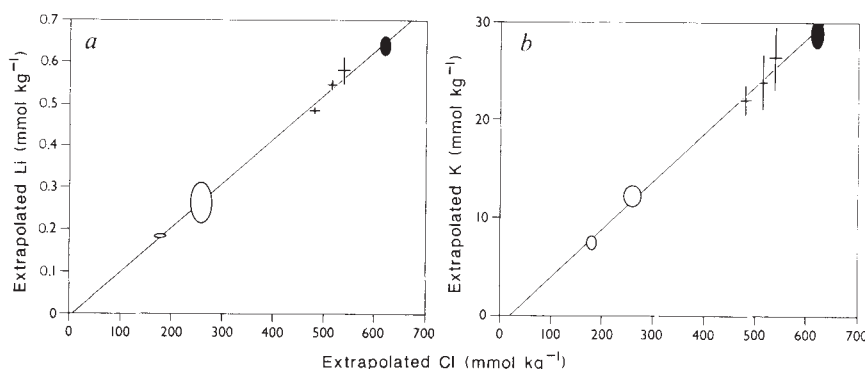


in boiling conditions and the extent of phase segregation precludes strict comparison of our results to those in ref. 17, the trend and magnitude of gas partitioning at ASHES is very suggestive of phase separation.

Comparison of the Cl-depleted and Cl-enriched endmember values for Cl, Na, K, Br and Li yields a remarkably constant ratio of  $0.293 \pm 0.029$ . For Ca, however a 27% relative deficiency

results. If we assume that the missing Ca has been removed by anhydrite precipitation during mixing of hydrothermal fluids with a small amount of cold sea water then the estimated composition of the Cl-depleted endmember is: 71% pure condensed vapour, 24% Cl-enriched fluid and 5% sea water. Application of a boiling fractionation model<sup>17</sup> to fluids initially at seawater chlorinity yields Inferno-level chloride concentrations

FIG. 3 *a*, Extrapolated Li (mmol kg<sup>-1</sup>) and *b*, extrapolated K (mmol kg<sup>-1</sup>) plotted against extrapolated Cl (mmol kg<sup>-1</sup>) for high-temperature vents at ASHES. For *a*,  $y = -0.006 + 0.00105x$ ; correlation coefficient  $r = 0.998$ . For *b*,  $y = -0.83 + 0.49x$ ;  $r = 0.997$ . Vent symbols in order of increasing value: Virgin Mound, Crack Vent (both open symbols); Hillock, Mushroom, Hell (all crosses); Inferno (closed symbol). Discrete values obtained by extending lines formed between Li-Mg, K-Mg and Cl-Mg data pairs for sea water and sample to respective zero Mg concentrations. The average (centre point of symbols) and standard deviation (height and width of symbols =  $2\sigma$ ) of pooled discrete values for each vent are shown. This presentation removes the seawater component from the sample mixtures and demonstrates for these species, which are conservative during seawater mixing, that all of the high-temperature fluids venting at ASHES can be described by a single mixing line that has, as one of its



endmembers, fresh water like that expected to comprise the vapour phase during sub-critical phase separation<sup>20</sup>.

after removal of ~20% of the water to the vapour phase, suggesting that Inferno fluids may be representative of a brine resulting from sub-critical phase separation (D.A.B. *et al.*, submitted). We note, however, that fluids with chlorinities both higher and lower than the extremes we have sampled may exist in the subsurface and that the magnitude of the Inferno Cl-enrichment could also be the result of fluid-rock interaction. Comparison of the venting distribution pattern to the self-consistent mixing behaviour of the ASHES fluids (Fig. 3) suggests that the venting endmembers are physically separated from one another well below the sea floor. The presence of Cl-depleted fluids within warm vents is consistent with studies of petroleum/gas reservoirs<sup>23</sup> and terrestrial hydrothermal systems<sup>24</sup>, for which differential flow mechanics have been used to successfully model spatial segregation of flow phases (C. Fox, submitted).

The unexpectedly high Si(OH)<sub>4</sub>, Ge and B values in the Cl-depleted fluids can be attributed to fluid-mineral reaction between the ascending vapour-enriched fluid and wall substrate and/or the high acid volatilities of these elements. Based on observations of B in geothermal systems<sup>25</sup>, however, the expected contributions as a result of acid volatility would be insufficient to account for the observed levels in the Cl-depleted fluids, implying that fluid-mineral reaction is the major source of the enrichments. The Si(OH)<sub>4</sub> values for both endmembers are slightly supersaturated with respect to quartz with concentrations and temperatures suggesting an equilibration depth of 290 bar or 1.4 km below the sea floor assuming hydrostatic pressure<sup>6</sup>. Based on the CO<sub>2</sub> content of vesicles within Axial Volcano basalts, Dixon *et al.*<sup>26</sup> estimated that a magma chamber lay 2.7 km below the sea floor. Although these depth estimates may be poorly constrained, as is the depth of the proposed phase separation, a reaction path capable of producing the Si(OH)<sub>4</sub>, Ge and B enrichments can be easily envisioned.

Phase-separation of hydrothermal fluids may have important consequences regarding subsurface ore deposition and delivery of metals to the deep ocean. The fact that regional hydrothermal plumes do not have as strong a metalliferous signature as plumes sampled elsewhere along the Juan de Fuca ridgecrest<sup>27</sup> may reflect the unusual source chemistry at ASHES. □

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## U-Pb baddeleyite ages for the Scourie dyke swarm, Scotland: evidence for two distinct intrusion events

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PRECISE and accurate radiometric ages for continental mafic dyke swarms are a prerequisite for global correlation of mafic magmatic events, calibration of apparent polar-wander paths and deciphering mechanisms of dyke emplacement. Precambrian dyke swarms, such as the Scourie dyke swarm in north-west Scotland, are invaluable time-markers, so that a precise and accurate knowledge of their emplacement age is critical when unravelling the complex geological evolution of many terrains. Precise dating is often difficult, however, because magma interaction with country rock and subsequent metamorphic events can severely perturb some isotopic systems. Recent advances in U-Pb geochronology<sup>1-3</sup> combined with the discovery that some mafic dykes contain trace amounts of uranium-bearing minerals such as baddeleyite (ZrO<sub>2</sub>) and/or zircon have made it possible to obtain U-Pb ages for these rocks with a precision typically on the order of 1-2 Myr<sup>4,5</sup>. Some

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of the first detailed U–Pb studies of Precambrian mafic dyke swarms<sup>5,6</sup> have shown that large volumes of mafic magma, 100,000 km<sup>3</sup>, were emplaced into the continental crust in surprisingly short periods of time (<2 Myr). Here we report the results from a U–Pb study of three members of the Scourie dyke swarm, and find at least two periods of dyke emplacement at 2,418 and 1,992 Myr BP. We speculate on a potential global correlation of early Proterozoic mafic magmatism and hence on the origin of dyke swarms.

The Scourie dyke swarm consists of numerous, 10–100 m wide, mafic to ultramafic dykes that intrude the high-grade granulite- and amphibolite-facies gneisses of the Archaean Lewisian complex in north-west Scotland<sup>7–9</sup>. Four distinct dyke suites can be recognized based on petrological and geochemical criteria: Mg-rich bronzite picrites, norites, olivine gabbros and Fe-rich quartz dolerites, the latter being most abundant. From field evidence, the dolerites are most often older than the picrite, norite and olivine gabbro suites. But some dolerites do post-date the latter<sup>7–9</sup>. Unfortunately, field evidence allows only a very small proportion of the dykes to be placed in a relative chronological sequence. The dykes were emplaced between the late-Archaean Scourian tectonometamorphic events and the mid-Proterozoic Laxfordian events, which are characterized by strong shear deformation. In the central zone of the Lewisian complex, the granulite-facies metamorphism peaked at ~2.7 Gyr, biotite-pegmatites were emplaced at ~2.5 Gyr, followed by localized retrogression of the gneisses to amphibolite-facies assemblages (Inverian event)<sup>10–12</sup>. Most of the Scourie dykes were emplaced penecontemporaneously with this hydrous retrogressive phase, and many were affected by it, as well as by later Laxfordian metamorphism. The emplacement of the Scourie dyke swarm is therefore important in understanding the timing of the Scourian and Inverian events.

Because of its geological significance, the Scourie dyke swarm has been the subject of numerous geochronological investigations<sup>13–15</sup> and, until recently, a relatively precise Rb–Sr whole-rock age of 2,390 ± 20 Myr (ref. 13), based on samples from three quartz dolerite samples, was considered to be the emplacement age for the swarm. Cohen *et al.*<sup>15</sup> challenged the validity of this Rb–Sr age, however, and proposed that the swarm is >300 Myr younger than this age, based on Sm–Nd internal isochrons for three relatively fresh dyke samples.

We collected large (≥30 kg) samples from many fresh dyke localities (some of these outcrops were sampled in the initial K–Ar study<sup>14</sup>) and most were searched intensively for baddeleyite and zircon. We took samples of all four petrogenetic types listed above and for most of these we sampled the coarser-

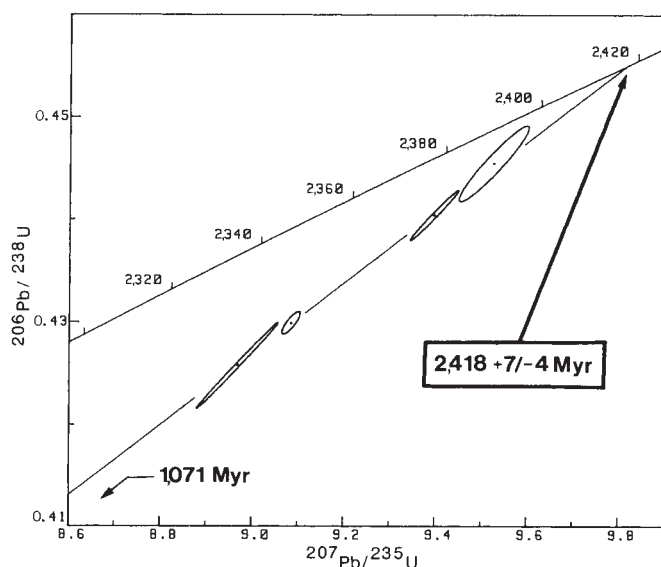


FIG. 1 U–Pb concordia diagram showing the results for four baddeleyite fractions from a member of the bronzite picrite suite (Beannach dyke).

grained central portions of the dykes. We found that three samples, representing members of the bronzite picrite, norite and olivine gabbro suites, contained sufficient quantities of baddeleyite/zircon for a U–Pb study. Two of these samples contained baddeleyite; a bronzite picrite (Beannach Dyke<sup>12</sup>) from a location 1 km west of Loch Assynt and an olivine gabbro from a dyke located 0.5 km south-west of Strathan (referred to here as the Strathan Dyke). At the Beannach Dyke sample locality, the dyke shows a primary petrological variation from orthopyroxene-rich margins to a plagioclase-rich centre<sup>12</sup> and, although different parts of the dyke were sampled to encompass the range of compositional variation, baddeleyite was only found in the central part, which is notably enriched in late-stage liquid. The olivine gabbro dykes typically show clinopyroxene-enriched margins, but baddeleyite was again found in the central portion of the Strathan Dyke. The zircon-bearing sample is a norite (N<sub>1</sub> of ref. 16) from Badcall Bay.

The U–Pb results for the baddeleyite and zircon fractions that were separated from these three samples are listed in Table 1 and are shown on concordia diagrams (Figs 1–3). As commonly

TABLE 1 U–Pb results for accessory minerals separated from three Scourie dykes

| Description                      |                   | Concentration  |                                 | Sample/Total<br>common<br>Pb(μg) | Atomic ratios*                          |                                         |                                        |                                        |                                         | Apparent age (Myr)                     |                                        |                                         |       |
|----------------------------------|-------------------|----------------|---------------------------------|----------------------------------|-----------------------------------------|-----------------------------------------|----------------------------------------|----------------------------------------|-----------------------------------------|----------------------------------------|----------------------------------------|-----------------------------------------|-------|
| Sample<br>Number                 | †Fraction         | weight<br>(μg) | U<br>(p.p.m.)<br>Pb<br>(p.p.m.) |                                  | <sup>206</sup> Pb/<br><sup>204</sup> Pb | <sup>208</sup> Pb/<br><sup>206</sup> Pb | <sup>206</sup> Pb/<br><sup>238</sup> U | <sup>207</sup> Pb/<br><sup>235</sup> U | <sup>207</sup> Pb/<br><sup>206</sup> Pb | <sup>206</sup> Pb/<br><sup>238</sup> U | <sup>207</sup> Pb/<br><sup>235</sup> U | <sup>207</sup> Pb/<br><sup>206</sup> Pb |       |
| Bronzite picrite (Beannach dyke) |                   |                |                                 |                                  |                                         |                                         |                                        |                                        |                                         |                                        |                                        |                                         |       |
| 1                                | B, 5M, br         | 1              | 1,838                           | 816                              | 6                                       | 86,635                                  | 0.0029                                 | 0.4455 (9)                             | 9.526 (18)                              | 0.15509 (10)                           | 2,375                                  | 2,390                                   | 2,403 |
| 2                                | B, 5M, br, (30)   | 6              | 2,476                           | 1,089                            | 25                                      | 20,307                                  | 0.0038                                 | 0.4404 (6)                             | 9.396 (14)                              | 0.15474 (3)                            | 2,352                                  | 2,377                                   | 2,399 |
| 3                                | B, 5M, br, (17)   | 3              | 1,777                           | 766                              | 11                                      | 23,566                                  | 0.0228                                 | 0.4257 (21)                            | 8.968 (44)                              | 0.15278 (5)                            | 2,287                                  | 2,335                                   | 2,377 |
| 4                                | B, 5M, br, (28)   | 3              | 1,652                           | 715                              | 6                                       | >100,000                                | 0.0175                                 | 0.4299 (5)                             | 9.085 (9)                               | 0.15326 (11)                           | 2,305                                  | 2,347                                   | 2,383 |
| Olivine gabbro (Strathan dyke)   |                   |                |                                 |                                  |                                         |                                         |                                        |                                        |                                         |                                        |                                        |                                         |       |
| 5                                | B, 5M, br, (16)   | 2              | 1,705                           | 604                              | 22                                      | 4,530                                   | 0.0045                                 | 0.3611 (4)                             | 6.094 (7)                               | 0.12241 (9)                            | 1,987                                  | 1,989                                   | 1,992 |
| 6                                | B, 5M, br, (18)   | 2              | 1,744                           | 617                              | 38                                      | 2,402                                   | 0.0064                                 | 0.3559 (4)                             | 6.004 (6)                               | 0.12236 (7)                            | 1,963                                  | 1,977                                   | 1,991 |
| 7                                | B, 5M, br, (31)   | 2              | 1,341                           | 467                              | 4                                       | >100,000                                | 0.0095                                 | 0.3581 (8)                             | 6.041 (12)                              | 0.12234 (14)                           | 1,973                                  | 1,982                                   | 1,991 |
| Norite, Badcall Bay              |                   |                |                                 |                                  |                                         |                                         |                                        |                                        |                                         |                                        |                                        |                                         |       |
| 8                                | Z, 3M, c, ab, (7) | 2              | 376                             | 96                               | 29                                      | 436                                     | 0.1273                                 | 0.2103 (5)                             | 3.919 (9)                               | 0.13517 (12)                           | 1,230                                  | 1,617                                   | 2,166 |
| 9                                | Z, 3M, c, ab      | 2              | 763                             | 188                              | 33                                      | 737                                     | 0.1043                                 | 0.2151 (3)                             | 4.038 (6)                               | 0.13616 (10)                           | 1,256                                  | 1,642                                   | 2,179 |
| 10                               | R, 3M, gb, ab     | 48             | 12                              | 4                                | 48                                      | 267                                     | —                                      | 0.2896 (21)                            | 4.154 (38)                              | 0.10402 (63)                           | 1,640                                  | 1,665                                   | 1,697 |
| 11                               | R, 3M, or, ab     | 32             | 21                              | 8                                | 75                                      | 189                                     | —                                      | 0.2888 (18)                            | 4.125 (43)                              | 0.10359 (81)                           | 1,636                                  | 1,659                                   | 1,689 |

\* Atomic ratios corrected for blank (Pb = 5 μg; U = 2 μg) and initial common Pb (ref. 19). All errors are quoted at 1σ and have been propagated to include most sources of uncertainty.

† Mineral analysed: B, baddeleyite; Z, zircon; R, rutile. Magnetic susceptibility: N, non-magnetic; M, magnetic at the indicated angle of side tilt on a Frantz isodynamic separator. For example, 3M refers to a magnetic fraction at three degrees side tilt. Colour: c, colourless; br, brown; gb, grey-brown; or, orange, ab, abrasion treatment<sup>3</sup>. A number in parentheses corresponds to the total number of grains analysed.

found in mafic dyke samples, the yield and grain size (longest dimension between 20–80  $\mu\text{m}$ ) of zircon and baddeleyite in the Scourie dyke samples were small and hence the weights of the fractions analysed (1–6  $\mu\text{g}$ , Table 1) are small. The analytical procedures that we used for isolating U and Pb from baddeleyite and zircon and for measuring the U and Pb isotopic compositions on a VG354 mass spectrometer generally followed those in refs 2, 6 and 17.

The U–Pb results for four baddeleyite fractions from the Beannach Dyke are shown in Fig. 1. These four fractions define a discordia line (27% probability of fit) giving an upper-intercept age of  $2,418 \pm 7/-4$  Myr ( $2\sigma$ ) and a lower-intercept age of 1,071 Myr. A striking feature of these baddeleyite analyses is the amount of discordance present (3.5–10.2%). The Beannach Dyke baddeleyite is unusual, however, in that many grains have a thin (5–20  $\mu\text{m}$  thick) rim of polycrystalline zircon similar to that observed in some coronitic gabbros<sup>18</sup> and it is the presence of tiny amounts of zircon rim material that is largely responsible for the degree of baddeleyite discordance. Fractions 3 and 4 (Table 1), which show the greatest amount of discordance, contained baddeleyite grains with some visible zircon rim material. Furthermore, these same two fractions have noticeably higher  $^{208}\text{Pb}/^{206}\text{Pb}$  ratios, reflecting a higher Th content in the zircon rim material. Unfortunately, the zircon rims are too thin to analyse separately so it is not possible to ascertain whether they are magmatic or a product of later metamorphism. A magmatic origin is perfectly feasible because the dyke is particularly fresh at this locality and it is also the most Zr-rich facies of the dyke. If the zircon rims are magmatic, the 2,418-Myr age represents the emplacement age of the dyke. If they are metamorphic, the emplacement age could be slightly older, to an extent depending on the age of zircon growth and the subsequent Pb-loss history.

The U–Pb results for the baddeleyite-bearing olivine gabbro dyke at Strathan are presented in Fig. 2. The three baddeleyite fractions (5–7, Table 1) have similar  $^{207}\text{Pb}/^{206}\text{Pb}$  ages, between 1,992 and 1,991 Myr, and are between 0.3 and 1.8% discordant. No visible zircon rim material is present in this baddeleyite population. A best-fit discordia line through these analyses gives an upper-intercept age of  $1,992 \pm 3/-2$  Myr (78% probability of fit) and we interpret this as the emplacement age for this dyke.

We separated both zircon and rutile from the  $\text{N}_1$  norite at Badcall Bay and the U–Pb results for two fractions of each mineral are shown in Fig. 3. The  $^{207}\text{Pb}/^{206}\text{Pb}$  ages for the two

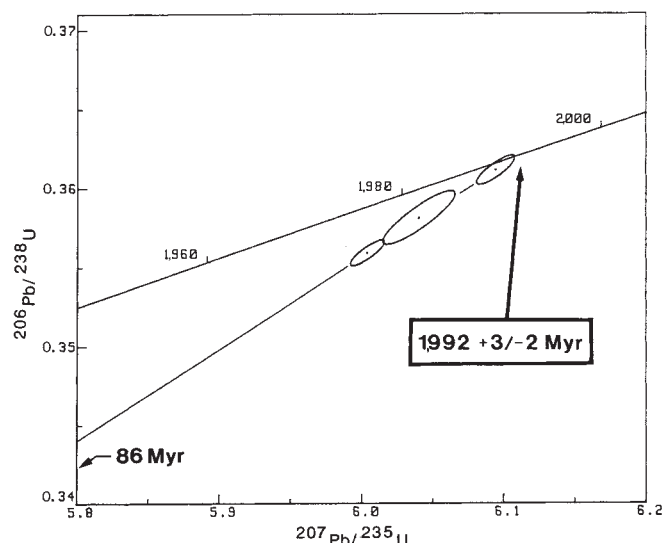


FIG. 2 U–Pb concordia diagram showing the results for three baddeleyite fractions from a member of the olivine gabbro suite (Strathan dyke).

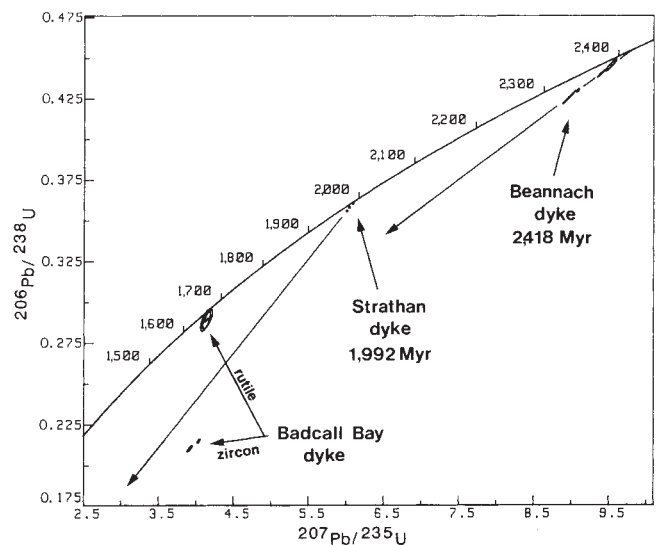


FIG. 3 Composite U–Pb concordia diagram showing the results for three Scourie dyke samples. Also shown are reference discordia lines for the Beannach and Strathan dykes with upper-intercept ages of 2,418 and 1,992 Myr, respectively.

zircon fractions are 2,166 and 2,179 Myr, respectively (fractions 8 and 9, Table 1) and provide a minimum estimate for the emplacement age of this dyke. Unfortunately, these two analyses are very discordant ( $>50\%$ ; see Fig. 3), even though they were given an air abrasion treatment<sup>3</sup>, so an accurate U–Pb zircon age cannot be established. We note, however, that the zircon fractions have suffered a significant amount of Pb-loss during a relatively recent event. Assuming the emplacement age for the norite dyke is identical to the Beannach Dyke ( $\sim 2,420$  Myr), with which it has petrogenetic affinity, then the projected lower-intercept age is  $\sim 440$  Myr. A possible cause for the substantial amount of Pb-loss in the two zircon fractions is tectonic disturbance related to early movements along the Caledonian Moine thrust zone. Many olivine-bearing dykes close to this thrust zone have in fact suffered late-stage serpentinization, indicating fluid activity at this time.

The two rutile fractions from the fresh Badcall Bay norite are also shown in Fig. 3 and have  $^{207}\text{Pb}/^{206}\text{Pb}$  ages of 1,697 and 1,689 Myr. Considering the nearly concordant nature of these analyses, it is evident that rutile was either formed, or the U–Pb systematics reset, after emplacement of even the youngest known Scourie dyke at 1,992 Myr. In either case, the  $\sim 1,700$ -Myr rutile age is clearly the result of a later Laxfordian metamorphic overprint. We note that the norite dyke is situated much closer to the Laxford metamorphic front than the olivine gabbros and picrites, which are located 15–20 km south of the front. Moreover, an extension of this norite ( $\text{N}_2$ ) only 30 m away, is completely altered and is cross-cut by a Laxfordian muscovite pegmatite.

These U–Pb baddeleyite results provide the first convincing evidence for at least two distinct episodes of Scourie dyke emplacement. They demonstrate that caution should be exercised in assuming that all dykes in the same location with similar orientations have identical emplacement ages. The results from previous petrogenetic studies<sup>9,12</sup> indicate that the norites and picrites have been derived from the same, rather refractory, mantle source whereas the olivine gabbros and quartz dolerites have been derived from a separate, more Fe-rich, mantle source. The U–Pb data are consistent with this idea because the bronzite picrite suite and probably the norite suite are part of an older dyke emplacement ( $\sim 2,418$  Myr) whereas the olivine gabbro suite was emplaced more than 400 Myr later, at 1,992 Myr.

The U–Pb minimum age of 2,418 Myr for the Beannach Dyke



is slightly older than the previously reported Rb–Sr whole-rock age of  $2,390 \pm 20$  Myr based on three dolerites from the Scourie area<sup>13</sup>, commonly accepted hitherto as the age of the entire swarm. If the Rb–Sr age was determined from pre-picrite dolerites then it may be regarded as anomalously young. The U–Pb baddeleyite age of 1,992 Myr for the Strathan Dyke generally agrees with the Sm–Nd mineral ages of 2,031–1,983 Myr determined for three separate fresh dolerite samples from the Scourie area<sup>15</sup>. Although the U–Pb results are generally consistent with both the previous Rb–Sr and Sm–Nd studies, the U–Pb baddeleyite results clearly demonstrate the presence of two temporally distinct periods of dyke intrusion in the Lewisian and resolves the controversy surrounding the previous age determinations. Unfortunately, the lack of baddeleyite in the fresh dolerites that we have sampled prevents any assessment by U–Pb techniques of the proportion of dolerites that might belong to the ~2400- or ~2000-Myr suites. In addition, the U–Pb results for accessory minerals from the norite dyke at Badcall Bay are evidence for disturbances in the U–Pb system related to both the later Laxfordian metamorphic episode (new growth or resetting of rutile) and Caledonian events (Pb-loss in zircon).

We stressed earlier that precise and accurate U–Pb baddeleyite ages for mafic dyke swarms are a prerequisite for global correlation of mafic magmatic events. Although precise ages are not yet available for most swarms, it is clear that mafic dyke swarms are a common feature of most Precambrian cratons worldwide with individual cratons containing numerous discrete dyke suites. One of the most promising techniques for correlating dyke swarms on a global scale is by using U–Pb baddeleyite geochronology. Interestingly, the 2,418-Myr age for the Scourie picrite suite is close to a recently determined U–Pb baddeleyite/zircon age of  $2,452 \pm 3/-2$  Myr for the Hearst–Matachewan swarm in North America<sup>5</sup>. Although correlation over such distances is necessarily speculative, we note that the least discordant results, from the Beannach Dyke baddeleyite that contained no visible zircon rim material, are also consistent with an emplacement age of ~2,450 Myr, with subsequent development of zircon rims during Laxfordian metamorphism (~1,800 Myr) and a small component of relatively recent Pb-loss in the zircon. If this correlation can be further substantiated, then the origin of such widespread mafic magmatism requires consideration of processes within the Earth that operate globally, rather than origins for all dyke swarms being totally dependent on local tectonic constraints. Future application of precise U–Pb geochronology combined with other detailed studies on a variety of aspects of mafic dykes including efforts to obtain 'primary' initial Sr–Nd–Hf–Pb isotope information on accurately-dated dykes may help to elucidate the origin, significance and extent of temporally related major Precambrian mafic dyke swarms. □

## High-resolution leaf-fossil record spanning the Cretaceous/Tertiary boundary

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**THEORIES** that explain the extinctions characterizing the Cretaceous/Tertiary (K/T) boundary<sup>1–3</sup> need to be tested by analyses of thoroughly sampled biotas. Palynological studies are the primary means for stratigraphic placement of the terrestrial boundary and for estimates of plant extinction<sup>4–12</sup>, but have not been combined with quantitative analyses of fossil leaves (megaflores). Megaflores studies complement palynology by representing local floras with assemblages capable of high taxonomic resolution<sup>13</sup>, but have previously lacked the sample size and stratigraphic spacing needed to resolve latest Cretaceous floral history<sup>5,14–18</sup>. We have now combined megaflores data from a 100-m-thick composite K/T boundary section in North Dakota with detailed palynological analysis. Here the boundary is marked by a 30% palynofloral extinction coincident with iridium and shocked-mineral anomalies and lies ~2 m above the highest dinosaur remains. The megaflores undergoes a 79% turnover across the boundary, and smaller changes 17- and 25-m below it. This pattern is consistent with latest Cretaceous climatic warming preceding a bolide impact.

The badlands of the Little Missouri River near Marmarth, southwestern North Dakota, expose extensive fossiliferous outcrops of the Upper Cretaceous (Maastrichtian) Hell Creek Formation and the lower Palaeocene Ludlow Member of the Fort Union Formation<sup>19–21</sup>. The Hell Creek is a 110-m-thick unit of sandstone and mudstone representing channel- and floodplain-soil deposits of a meandering fluvial system<sup>19</sup>. It is conformably overlain by the 100-m-thick Ludlow Member, which consists of lignite, lenticular and tabular sandstone bodies and laminated mudstone. The Ludlow represents fluvio-deltaic deposition on the western margin of the Cannonball seaway of Danian age<sup>20,21</sup>. The Hell Creek–Ludlow contact is placed at the base of the lowest lignite. Dinosaur remains are common in the Hell Creek; their highest recorded appearance is 1 m below the base of the Ludlow Member (Milwaukee Public Museum, locality 1056).

Megaflores remains ( $n = 11,503$ ), were collected at 87 localities from the base of the Hell Creek to near the top of the Ludlow, in a  $25 \times 65$ -km field area centred in Marmarth. For each locality, stratigraphic position was measured, sedimentary facies analysed and a census made to assess the relative abundance of megaflores taxa. Because of the chaotic state of Cretaceous angiosperm taxonomy, a morphotype system was established to assure reproducible identifications<sup>22</sup>. Megaflores data were plotted for each of the localities within 50 m of the formational contact that yielded >20 specimens ( $n = 8,428$ ; Fig. 1).

At Pyramid Butte, 12 km north of Marmarth, a 1.9-m section across the basal Fort Union lignite and adjacent strata was sampled in contiguous 10-cm intervals for neutron activation and palynological analyses (Fig. 2).

Neutron activation analyses revealed an iridium anomaly of 0.34 parts per  $10^9$  (p.p.b.) over background of 0.025 p.p.b. in a 10-cm sample including dark-grey mudstone and sandstone at the top of the 90-cm-thick lignite (Fig. 2). Subsequent sampling at 2–3-cm intervals showed an anomaly of 0.72 p.p.b. restricted to the mudstone. The mudstone also contains rare shock-metamorphosed mineral grains similar to those found at other K/T boundary sites<sup>9,23</sup>.

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Palynological analyses show that ~30% of the Upper Cretaceous palynoflora, including characteristic Maastrichtian taxa, is absent above the level of the iridium and shocked-mineral anomalies (Fig. 2). Palynomorph diversity decreases from ~90 taxa in the top metre of the Cretaceous strata to ~60 taxa in the basal 80 cm of the Palaeocene strata. The fern-spore abundance 'spike'<sup>10,24,25</sup> that characterizes several other K/T boundary sections was not found at Pyramid Butte, indicating either that it was not deposited, as seems to be the case in several sites in Canada<sup>26</sup>, or that it was subsequently removed. Minor disturbance of the boundary interval is suggested by the unconformable base of the overlying, 10-cm-thick, laterally discontinuous sandstone bed and common sand-filled burrows of, possibly, insects in the iridium-bearing mudstone and the top of the lignite. This may account for the small iridium anomaly, the relative rarity of shocked grains and the absence of the characteristic 'boundary clay layer'<sup>8,23</sup>.

Significantly, the palynologically defined K/T boundary and associated anomalies at Pyramid Butte occur above the basal lignite, some 90 cm into the Fort Union Formation. At all other published K/T sites in the northern Great Plains<sup>9,10,12</sup>, the boundary occurs at, or just below, the base of a lignite bed. This relationship was interpreted as evidence of an impact-related increase in precipitation and the resulting deposition of peat<sup>18</sup>. The Pyramid Butte site shows that the Hell Creek-Ludlow facies

transition began before the K/T transition, and implies different causes for the regional start of peat deposition and the K/T extinctions.

The megafloral data from Marmarth represent the first quantitative distribution of megaflora into zones in uppermost Cretaceous strata. Three zones are described here, two from the upper 50 m of the Hell Creek Formation and one from the basal 50 m of the Fort Union Formation (Fig. 1). The lowest, HC II, is divided into two subzones. The lower subzone, HC IIa, is dominated by *Dryophyllum subfalcatum* (43%) and *Platanophyllum montanum* (13%). Total diversity is 80 taxa (2,047 specimens, 12 localities). The overlying subzone, HC IIb, is dominated by '*Vitis*' *stantonii* (66%) and *D. subfalcatum* (14%). Total diversity is 12 taxa (1,044 specimens, 6 localities).

Only 25% of the HC II taxa persist into the uppermost Hell Creek zone, HC III, but much of this change is due to gradual turnover within HC II. Dominant taxa in HC III are *Dombeyopsis trivialis* (29%) and '*Cissus*' *marginata* (15%) (Fig. 1). Total diversity is 63 taxa (1,741 specimens, 17 localities). The HC III megaflora is unusual in that it contains many taxa previously known from the Upper Cretaceous of the Raton basin, 1,000 km to the south<sup>17,27</sup>. Their presence in North Dakota probably represents northward migration of a thermophilous flora (including palms and Laurales) in response to a warming climate. Latest Cretaceous climatic warming is further supported

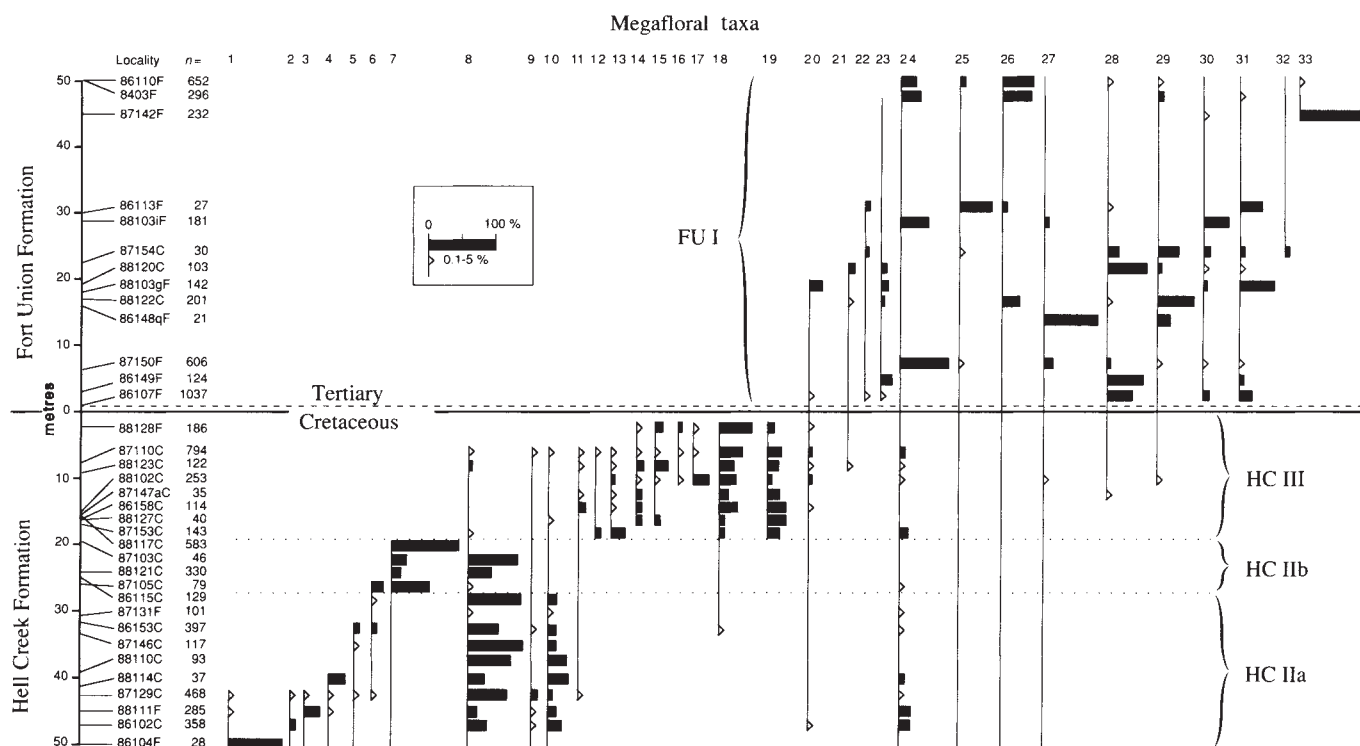


FIG. 1 Stratigraphic ranges and relative percentage histograms of megafloral taxa relative to the Hell Creek-Fort Union contact (solid line), the palynologically defined K/T boundary (dashed line) and megafloral zone boundaries (dotted lines). All taxa that account for 5% or more of the flora at any level and occur at two or more levels are plotted. The facies of each locality is noted by letter: C, channel, F, floodplain. Range lines that terminate with no tick marks represent taxa from Marmarth that occur outside the stratigraphic range of this chart or that occur at localities not plotted. Localities 86115 and 86117 are plotted out of stratigraphic sequence because they occur in a section that appears to have been compressed by a local hiatus. The taxa (and morphotype reference numbers) are: 1-*Pistia corrugata* (HC77), 2-*Cercidiphyllum ellipticum* (HC73), 3-*Ginkgo adiantoides* (HC114), 4-'*Ficus*' *artocarpoides* (HC86), 5(-aff.)Magnoliaceae (HC66), 6(-aff.)Dilleniaceae (HC62), 7-'*Vitis*' *stantonii* (HC14, HC108), 8-'*Dryophyllum*' *subfalcatum* (HC49), 9-*Trochodendroides nebrascensis* (HC103), 10-*Platanophyllum mon-*

*tanum* (HC57), 11-(aff.)Platanaceae (HC2), 12-*Paleoaster iniquiendi* (HC7), 13-(cf.)'*Dryophyllum*' *tennessensis* (HC44), 14-*Paranymphaea hastata* (HC111), 15-Laurales (HC162), 16-Laurales (HC163), 17-'*Liriodendron*' *laramiense* (HC166), 18-*Dombeyopsis trivialis* (HC105), 19-'*Cissus*' *marginata* (HC1, HC106), 20-Palmaceae (FU37), 21-(aff.)Hamamelididae (FU76), 22-Flacourtiaceae (FU49), 23-(aff.)Cornaceae (FU31), 24-Taxodiaceae including *Glyptostrobus europaeus* (FU4, HC9), *Metasequoia occidentalis* (FU3, HC35), *Taxodium olrikii* (HC71), *Sequoia* sp. (HC70), and unidentified taxodiaceous foliage, 25-*Cercidiphyllum genetrix* (FU5), 26-'*Cocculus*' *flabella* (FU43), 27-*Cupressinocladus interruptus* (FU26) 28-'*Populus*' *nebrascensis* (FU7), 29-*Platanus raynoldsii* (FU16), 30-*Paranymphaea crassifolia* (FU1), 31-*Dicotylophyllum anomalum* (FU29), 32-unidentified dicotyledon (FU47), 33-*Quereuxia angulata* (FU2). Aff., affinity (implies similarity); cf., compare (implies a closer similarity).

by physiognomic analysis of these megafloras that shows an increased percentage of dicotyledonous angiosperms with entire-margined leaves (from 35% to 49%) on transition from HC IIa to HC III (ref. 28).

Megafloral turnover at the K/T boundary in southwestern North Dakota is extremely high and seems to represent major extinction. The HC III megaflora is replaced by basal Palaeocene megaflora FU I. Because pollen and leaves are derived from the same vegetation, megafloral change is probably as abrupt as that represented by the palynoflora. Because similar facies (channel, floodplain) above and below the K/T boundary produce different mega- and palynofloras, and Cretaceous palynomorphs occur throughout the basal Fort Union lignite, it is unlikely that floral change is an artefact of facies or collecting bias (Fig. 2). Only 21% of the HC III taxa, including no dominants, survived into the Palaeocene. Zone FU I extends from Alberta to Colorado<sup>15,29-31</sup>. Near Marmarth it begins at the K/T boundary and is dominated by taxodiaceous conifers (20%), '*Populus nebrascensis*' (16%), '*Cocculus flabella*' (12%), '*Dictylophyllum anomalum*' (9%), and '*Paranymphaea crassifolia*' (5%). The total diversity is 72 taxa (3,795 specimens, 30 localities). The lowest FU I locality (86107) occurs 15 cm above the iridium anomaly at Pyramid Butte and has a diversity of 29 megafloral taxa (1,037 specimens). Consequently, prolonged existence of a low-diversity, post-impact, 'recovery' flora<sup>17,18</sup> seems unlikely.

For both palynoflora and megaflora, the K/T boundary marks the most distinct change in the section. Although the palynologi-

cal analysis concentrated on the 1.9 m boundary interval, other data from the Hell Creek indicate that the entire formation lies within the *Wodehouseia spinata* Assemblage Zone<sup>32</sup>. Subdivisions of this zone paralleling megafloral zones HC IIa-HC III are not clearly evident. These differences probably result from different taxonomic resolution and taphonomic pathways of the plant organs<sup>13</sup> and support an integrated approach based on the micro-stratigraphic resolution of palynomorphs and the high taxonomic resolution of megaflora. The disappearance of the uppermost Cretaceous megaflora, and its replacement by the widespread basal Palaeocene megaflora, in context with abrupt palynological extinctions, shocked minerals, and the iridium anomaly, supports the theory that floral extinctions at the K/T boundary were caused by the impact of an extraterrestrial body. □

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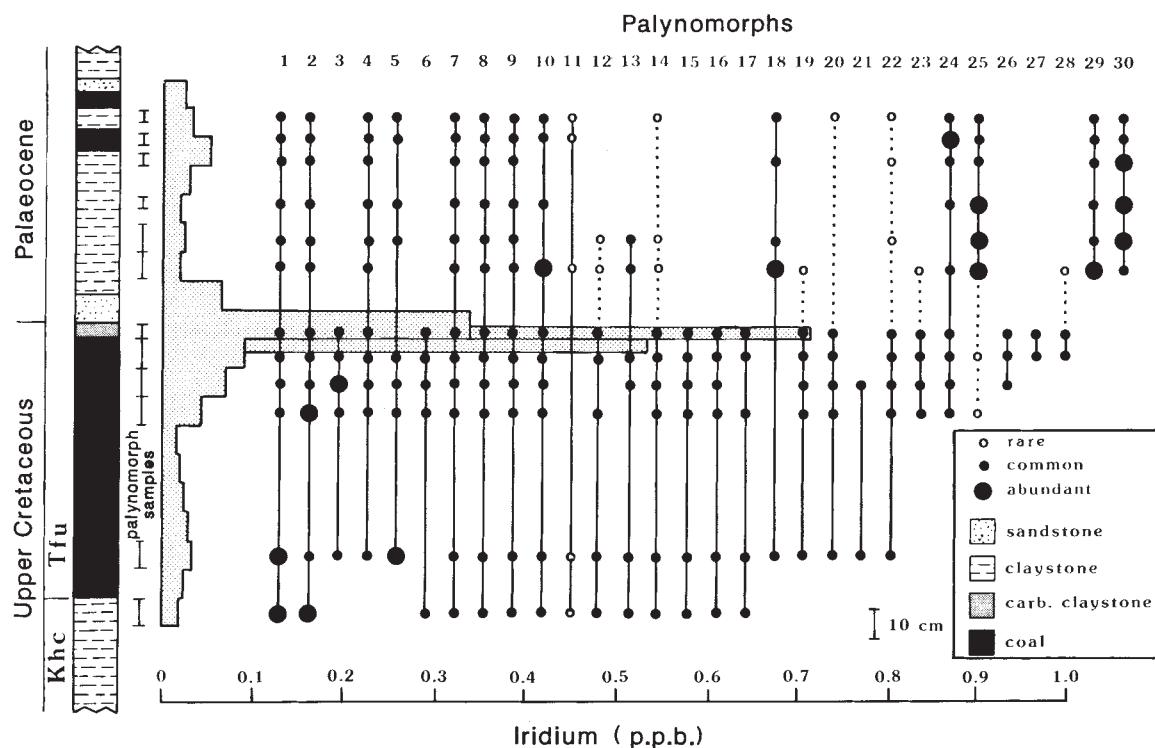


FIG. 2 Distribution and relative abundances of iridium and the most common palynomorphs in the 1.9-m-thick K/T boundary section at Pyramid Butte, North Dakota. Khc, Hell Creek Formation; Tfu, Fort Union Formation (Ludlow Member); histogram shows iridium concentrations in p.p.b. measured in continuous 10-cm-interval samples and the maximum anomaly from additional samples at smaller intervals; peak iridium anomaly is coincident with shocked mineral occurrence and disappearance of characteristic Upper Cretaceous palynomorphs; vertical lines show ranges of palynomorphs within sampled intervals; dotted lines extend ranges on basis of very rare, non-typical (possibly reworked) occurrences. Palynomorphs are grouped by taxonomic category: bryophytes and pteridophytes, 1-3; gymnosperms, 4-6; angiosperms, 7-30. The taxa are: 1-*Laevigatosporites* spp., 2-*Stereisporites*

spp., 3-*Osmundacidites* sp., 4-*Taxodiaceapollenites hiatus*, 5-(cf.)*Glyptosporobolus* sp., 6-*Ephedra multipartita*, 7-*Arecipites tenuixinous*, 8-*Tripopolitenites* spp., 9-*Ulmoidipites* spp., 10-*Kurtzipites trispissatus*, 11-*Momipites inaequalis*, 12-*Tricolpites parvistriatus*, 13-*Wodehouseia spinata*, 14-*Proteacidites* spp., 15-*Libopollis jarzenii*, 16-*Liliacidites complexus*, 17-*Myrtipites granulatus*, 18-*K. circularis*, 19-*Aquilapollenites* n. sp. 20-*A. quadrilobus*, 21-*Cranwellia rumseyensis*, 22-*T. microreticulatus*, 23-*Orbiculapollis lucidus*, 24-*T. hians*/parvus, 25-*Ericaceipollenites rullus*, 26-*A. delicatus* var. *collaris*, 27-*Leptococcolites pocockii*, 28-*A. reticulatus*, 29-*Pandaniidites typicus*, 30-*Syncolporites minimus*. n. sp., new species; sp., species; spp., more than one such species; var., variety.



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## Orb-web weaving spiders in the early Cretaceous

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THE use of a snare woven from spun silk as a means of capturing prey is the most outstanding achievement of spiders. Fossil spider spinnerets are known from the Devonian<sup>1</sup> and Carboniferous<sup>2</sup> periods. Presented here, however, is evidence of the antiquity of the use of woven silk in prey capture: spider fossils showing morphological adaptations for web weaving, from the Lower Cretaceous lithographic limestone of the Sierra de Montsec, north-east Spain. Reflected light microscopy reveals details of the pattern and structure of the tarsal claws, which were adapted for the handling of silk and locomotion on a web. As only two Mesozoic spiders, *Juraneus* and *Jurarchaea* from the Jurassic of the Soviet Union<sup>3</sup>, have been formally described, the four specimens reported here are an important addition to the fossil record. These belong to three new genera placed in the modern superfamilies Dinopoidea and Araneoidea. Members of both superfamilies weave orb webs or orb-web derivatives<sup>4</sup>. The Montsec spiders preyed on the abundant insect life which is also preserved in the Cretaceous lithographic limestone<sup>5</sup>.

The Lower Cretaceous lithographic limestone of the Sierra de Montsec, Lérida Province, north-east Spain, is renowned for its excellent preservation of land plants, crustaceans, insects, fish, amphibians, reptiles and birds<sup>5,6</sup>. Four specimens of spiders are among the collections made in recent years at the quarries of La Cabrua (specimens LC 1150 IEI, LC 1753 AP, LC 1754 AP) and La Pedrera de Meia (LP 1755 AP). Locality details are given in ref. 7 and specimens are deposited in the Instituto de Estudios Ilerdenses, Lérida. The fifty-metre succession of limestone exposed in the quarries has been determined as late Berriasian to early Valanginian in age on the basis of microfossils<sup>6,8</sup>. The fine-grained, thinly bedded limestones bearing the spider fossils represent a lagoonal or lacustrine environment between the Ebro continent to the south and marine conditions to the north<sup>6</sup>. The spiders are preserved as brittle, brown cuticle, and morphological details are seen best where the cuticle is covered by a thin layer of translucent matrix. Reflected light microscopy, using oil-immersion objectives, enables observation at high magnification. All three new genera belong to the suborder Araneomorphae; formal taxonomy of the new taxa will be published elsewhere<sup>9</sup> and only brief descriptions follow here.

LC 1150 IEI (Fig. 1) is referred to the Araneoidea. This superfamily is inadequately defined at present<sup>10</sup>, but features include: lack of synapomorphies of other groups, serrate setae, paracymbium, few trichobothria, web-weaving and globose abdomen. Although none of these characters are unique to Araneoidea, their combined presence in LC 1150 IEI suggest its inclusion in this superfamily. The subelliptical carapace bears a raised cephalic area and no fovea. The abdomen (globular in life) bears a compact group of short spinnerets at the posterior. The sternum is subtriangular and there is a small, subtriangular labium. Serrate setae are present and no trichobothria can be seen on the specimen. The chelicerae are large (0.4 times the carapace length), forwardly directed and bear inner and outer rows of denticles and a mesal ridge. The specimen is an adult male, bearing a modified palp with a long embolus and what appears to be a small, proximal paracymbium. The legs are

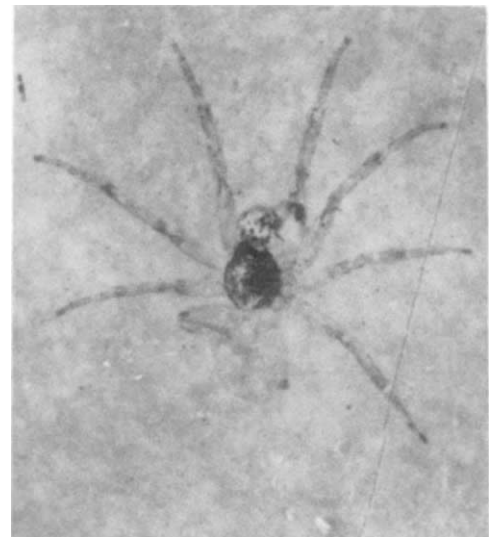


FIG. 1 Araneoid spider from the Lower Cretaceous of Montsec, Spain (LC 1150 IEI). Specimen compressed to the left; carapace offset to left, revealing right coxae; left male palp curved round anterior of left chelicera and showing long embolus, right palpal bulb lying on right leg 1; spinnerets appear as dark patch in lower right of abdomen (compressed to the left). Magnification,  $\times 5.2$ .

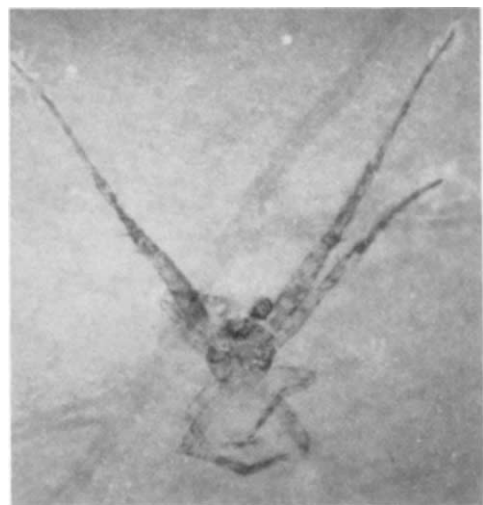


FIG. 2 Araneoid spider from the Lower Cretaceous of Montsec, Spain (LC 1753 AP). Note subcircular carapace, right male palp with planospirally coiled embolus, elongate legs 1 and 2, abdomen absent in this specimen. Magnification,  $\times 3.2$ .

subequal in length (formula, shortest to longest, 1243) and are about 3 times the length of the carapace. The tarsi bear pectinate paired claws, a small, non-pectinate median claw and numerous serrate bristles (Fig. 4a). It is impossible to assign the specimen to a family on these characters, but a small, sheet-web weaving araneoid (such as linyphiid or theridiid) is suggested.

LC 1753 AP (Fig. 2) and LC 1754 AP belong to the same species, which is referred to the Araneoidea. The foveate carapace is slightly wider than long and the oval abdomen bears a subterminal group of short spinnerets. Serrate setae occur and trichobothria are present on the superior prolateral surface of femora 3 (double row) and 4 (single row). The sternum is circular. Both specimens are adult males bearing modified palps with a planospirally coiled embolus. The leg formula is 1243, leg 1 being 6 times the length of the carapace and more than twice the length of leg 3. The paired tarsal claws bear six teeth, the median claw is long, curved and not pectinate and accessory claws are present (Fig. 4b). The pattern of elongated anterior legs, short third legs and femoral trichobothria occurs as a convergent phenomenon in two extant spider families: the Uloboridae (Dinopoidea) and the Araneidae (Araneoidea). Both groups are wrap-attack orb-web weavers, the former using cribellum and calamistrum to produce non-viscid 'cribellate' silk in contrast to the viscid silk of araneids. LC 1753 AP and LC 1754 AP lack characters that would refer them to the Dinopoidea (see below) and appear to be closest to the Argiopinae within the family Araneidae.

The carapace of LP 1755 AP (Fig. 3) is oval, has no well-defined fovea and just posterior to the midline is a break of slope which accommodated the forwardly extended abdomen in life. A compact group of six spinnerets and a cribellum is present subterminally on the abdomen. The leg formula is 1243; leg 1 is more than 5 times the length of the carapace and more than twice the length of leg 3. Plumose setae are present and there are many trichobothria on what appears to be the retrolateral surface of femur 2 and possibly the prolateral surfaces of femora 3 and 4. The paired tarsal claws are small and lack teeth, the median claw is long and probably lacks teeth (if

present, they are minute). A pair of large accessory claws is present on the tarsi. The superior surface of metatarsus 4 is curved and bears a calamistrum. The palps are unmodified, so this is a female or immature specimen. LP 1755 AP belongs in the superfamily Dinopoidea, possessing calamistrum and cribellum, plumose setae and a characteristic tarsal claw pattern; it lacks the tarsal macrosetae and feathery setae of uloborids<sup>11</sup>, and is therefore not placed in that family.

All three Montsech spider genera possess three tarsal claws with the characteristic serrate accessory claws adapted for web-weaving<sup>12</sup>. The combination of elongated anterior legs, short third legs and femoral trichobothria in LC 1753 AP, LC 1754 AP and LP 1755 AP occurs in only two groups of living spiders, Uloboridae and Araneidae, both of which are weavers of orb webs. These spiders rest in characteristic positions<sup>13</sup>, generally with the anterior legs outstretched, and the short third legs gripping a twig. The function of the femoral trichobothria is not understood, but the organs occur, as in the fossil spiders, on the retrolateral surface of forwardly directed femora and prolateral surfaces of backwardly directed femora. Thus, at rest, the trichobothria point to the lateral sides of the animal.

There is controversy over whether the orb web is a convergent phenomenon in Araneoidea and Dinopoidea, or whether it



FIG. 3 Dinopoid spider from the Lower Cretaceous of Montsech, Spain (LP 1755 AP). Specimen is dorsoventrally compressed, dorsal and ventral surfaces superimposed. Note elongate legs 1 and 2, dark line on superior (posterior in specimen) side of metatarsus 4 indicates calamistrum; abdomen (wrinkled posteriorly) with ventral group of small spinnerets, transverse line immediately anterior marks cribellum. Magnification,  $\times 7.4$ .

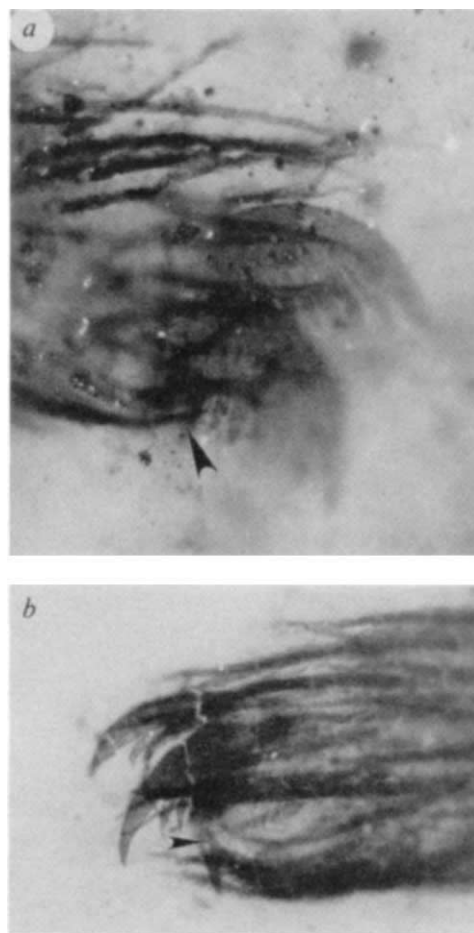


FIG. 4 a, Tarsal claws on left leg 2 of araneoid spider LC 1150 IEI. Note large, pectinate paired claws, small, uncinuate median claw (arrowed), serrate accessory claws (bottom) and bristles. Magnification,  $\times 250$ . b, Tarsal claws on right leg 2 of araneid spider LC 1753 AP. Note pectinate paired claws, median claw (arrowed), and large, serrate accessory claws (bottom) and bristles. Magnification,  $\times 250$ . In web-weaving spiders, the silken thread is pulled by the median claw on to the serrations of the accessory claws; the median claws take no part in normal handling of silk<sup>15</sup>. Spiders which do not weave prey-capture webs do not possess accessory claws, and the median claw may be absent or greatly reduced.



evolved only once<sup>10</sup>. The Montsech spiders provide evidence that the two groups of orb-web weavers were already well defined by the early Cretaceous. No tarsal claw details are preserved in the Jurassic araneoid *Juraneus*<sup>14</sup>; nevertheless, it may have been an orb-web weaver. If the orb web evolved only once, in the common ancestor of Araneoidea and Dinopoidea<sup>4</sup>, its origin lies in the Jurassic or earlier. □

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## Interspecific competition increases local extinction rate in a metapopulation system

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THE importance of interspecific competition for the distribution and abundance of organisms has been hotly debated during the last decade<sup>1–7</sup>. Although many field experiments have shown effects of interspecific competition on abundance and reproduction<sup>1,3</sup>, there is no unequivocal experimental evidence that interspecific competition can influence rates of local extinction in the field. Here I report that in a long-term field experiment with artificial rockpools, interspecific competition between three common rockpool zooplankton species led to increased local extinction rates. In addition, studies of the distributional dynamics of the species in natural rockpools also showed that interspecific competition increases extinction rates. These results imply that interspecific competition is likely to limit the regional richness of species in the rockpool metapopulation system. MacArthur and Wilson<sup>8</sup> were the first to suggest that an increase in extinction rate per species with an increase in the number of species could influence species richness on islands. Moulton and Pimm<sup>9</sup> found that this was so among birds introduced to the Hawaiian islands, but the present study is the first field experiment providing unequivocal evidence of the effect.

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Rockpools, small bedrock depressions containing fresh or brackish water, are common in coastal areas around Scandinavia. The most abundant small crustaceans in rockpools are three waterflea species, *Daphnia magna*, *D. pulex* and *D. longispina* (Cladocera)<sup>10–15</sup>, which coexist regionally along the coasts of Sweden<sup>11</sup> and Finland<sup>12–15</sup>. The smallest rockpools containing *Daphnia* have a volume of ~20 litres, and the largest ~10<sup>5</sup> litres. The niches of the species widely overlap along several habitat niche dimensions<sup>11,12</sup>, as well as the food axis<sup>11</sup>. Hanski and Ranta<sup>14</sup> proposed that the distributions of *Daphnia* in rockpools could be explained in terms of a colonization-extinction model, in which interspecific competition was assumed to influence extinction- and colonization rates. Such metapopulation models, where a metapopulation is defined as a regional population consisting of a number of patchily distributed local populations, have begun to play an important part in basic as well as applied ecology<sup>16,17</sup>, but there is little experimental or field evidence to support them.

The field experiment used the three *Daphnia* species mentioned<sup>10,11</sup>. It was conducted during 1983–86 and 1984–87 in artificial rockpools of four different sizes, namely 4-, 12-, 50- and 300-litre plastic bowls, placed outdoors and filled with freshwater. Experiments were run with the three-species combination, the three two-species combinations, and several one-species controls (Tables 1 and 2). Probably about sixteen, but at the very least eight, *Daphnia* generations occurred during the four-year experimental period<sup>11</sup>. In addition, species occurrences and environmental conditions were studied in more than 400 natural rockpools in three areas along the Swedish coast for five years (ref. 11; Tables 3 and 4).

Extinction rates (population<sup>-1</sup>. yr<sup>-1</sup>) were always higher in the three-species- than in the two-species experiments, and no extinctions occurred in the one-species controls (Table 1). Extinction rates were also greater the smaller the artificial pools (Table 1). Analysis of the results of all the 4-litre experiments, and those experiments that persisted until the end of the fourth

TABLE 1 Probabilities of extinction, average mean densities and average coefficients of variation in density of *Daphnia* populations in artificial rockpools

| Volume (litres) | Extinction probability (per population per year) ± s.d. |                         |                           | Average mean population density (individuals per litre) ± s.d. |                     | Average coefficient of variation in density ± s.d. |                     |
|-----------------|---------------------------------------------------------|-------------------------|---------------------------|----------------------------------------------------------------|---------------------|----------------------------------------------------|---------------------|
|                 | one-species experiments                                 | two-species experiments | three-species experiments | Persisting populations                                         | Extinct populations | Persisting populations                             | Extinct populations |
| 4               | 0 (—) (n=6)                                             | 0.21 (±0.078) (n=28)    | 0.28 (±0.11) (n=18)       | —                                                              | —                   | —                                                  | —                   |
| 12              | 0 (—) (n=3)                                             | 0.028 (±0.019) (n=22)   | 0.20 (±0.10) (n=12)       | 125* (±97.9) (n=22)                                            | 5.4* (±3.66) (n=5)  | 131 (±38.3) (n=22)                                 | 122 (±40.8) (n=5)   |
| 50              | 0 (—) (n=4)                                             | 0 (—) (n=26)            | 0.10 (±0.047) (n=15)      | 54.1* (±30.9) (n=26)                                           | 10.6* (±10.4) (n=4) | 143 (±37.1) (n=26)                                 | 188 (±64.7) (n=4)   |
| 300             | —                                                       | 0 (—) (n=2)             | 0.030 (±0.029) (n=9)      | 32.3 (±16.6) (n=10)                                            | 5.6 (—) (n=1)       | 159 (±38.5) (n=10)                                 | 137 (—) (n=1)       |

Experiments were performed during 1983–86 or 1984–87, except 4-l experiments, which lasted for one summer (1983 or 1984). Four (±1) replicates of each two-species combination and the three-species combination were run, except for 4-l pools where six three-species experiments were run, and for 300-l pools where only one two-species experiment was run. One-species controls, with at least one for each species, were run in 4-, 12- and 50-l pools. The experiments were inoculated with a natural pond phytoplankton assemblage in the middle of May the year of starting, and about two weeks later *Daphnia* from natural rockpools were introduced<sup>11</sup>. *Daphnia* survived winters as resting eggs. The results are based on samples from three to four sampling dates per year spaced evenly from May to autumn. Only established populations were used when calculating extinction probabilities. An extinction was judged to have occurred if an established species present in one year was absent in the following years. The 4-l pools were sampled and analysed in their entirety (including counts of resting eggs) at the end of the summer. Extinction probabilities were calculated on a population basis, regardless of species. The mean population densities and coefficients of variation in density for each population in each two- and three-species experiment were used for calculating averages in persisting and extinct populations. Some vessels broke during winters, and those present in only the first year were excluded from these analyses. For extinct populations, only values of population densities before extinction had occurred were used. Coefficients of variation were corrected for unequal sample sizes. See ref. 11 for further information and data. n, Initial number of populations; asterisk indicates significant difference between persisting and extinct populations (Mann-Whitney U Test,  $P < 0.01$ ).



TABLE 2 Number of extinctions of *Daphnia* species in artificial rockpools after a 4-year experimental period

| Volume<br>(litres) | Number of extinctions of <i>Daphnia</i> in different combinations |                  |                  |                                                        |                                                        |                                                        |                                                                                     |
|--------------------|-------------------------------------------------------------------|------------------|------------------|--------------------------------------------------------|--------------------------------------------------------|--------------------------------------------------------|-------------------------------------------------------------------------------------|
|                    | <i>M</i>                                                          | <i>P</i>         | <i>L</i>         | <i>M + P</i>                                           | <i>M + L</i>                                           | <i>P + L</i>                                           | <i>M + P + L</i>                                                                    |
| 4                  | 0 ( <i>n</i> =3)                                                  | 0 ( <i>n</i> =2) | 0 ( <i>n</i> =1) | <i>M</i> 0 ( <i>n</i> =4)<br><i>P</i> 2 ( <i>n</i> =4) | <i>M</i> 0 ( <i>n</i> =5)<br><i>L</i> 2 ( <i>n</i> =5) | <i>P</i> 0 ( <i>n</i> =5)<br><i>L</i> 2 ( <i>n</i> =5) | <i>M</i> 0 ( <i>n</i> =6)<br><i>P</i> 3 ( <i>n</i> =6)<br><i>L</i> 2 ( <i>n</i> =6) |
| 12                 | 0 ( <i>n</i> =1)                                                  | 0 ( <i>n</i> =1) | 0 ( <i>n</i> =1) | 0 ( <i>n</i> =2)                                       | 0 ( <i>n</i> =3)                                       | <i>P</i> 0 ( <i>n</i> =4)<br><i>L</i> 2 ( <i>n</i> =4) | <i>M</i> 2 ( <i>n</i> =3)<br><i>P</i> 0 ( <i>n</i> =3)<br><i>L</i> 1 ( <i>n</i> =3) |
| 50                 | 0 ( <i>n</i> =1)                                                  | 0 ( <i>n</i> =2) | 0 ( <i>n</i> =1) | 0 ( <i>n</i> =1)                                       | 0 ( <i>n</i> =4)                                       | 0 ( <i>n</i> =3)                                       | <i>M</i> 0 ( <i>n</i> =3)<br><i>P</i> 3 ( <i>n</i> =3)<br><i>L</i> 1 ( <i>n</i> =3) |
| 300                | —                                                                 | —                | —                | —                                                      | 0 ( <i>n</i> =1)                                       | —                                                      | <i>M</i> 0 ( <i>n</i> =3)<br><i>P</i> 1 ( <i>n</i> =3)<br><i>L</i> 0 ( <i>n</i> =3) |

*n*, Number of populations of each species in pools persisting for four years (in 4 litres for one year). Values of *n* differ between species combinations because several bowls broke during winters. *Daphnia magna* (*M*), *D. pulex* (*P*) and *D. longispina* (*L*). In one 4-l and one 50-l experiment, *D. pulex* and *D. longispina* went extinct at about the same time. There were significantly more extinctions in three-species than in two-species experiments ( $P=0.00484$ ). Because of low values of *n* and a large number of pools with no extinctions, conventional statistical tests were not possible. Instead, the numbers of extinctions in two- and three-species experiments at the end of the experimental period in each of the four volumes were analysed using separate Fisher's exact tests. A procedure was then attempted that corresponded to Fisher's combined probability test<sup>23</sup>, but because the exact tests had discrete, not continuous, probability distributions, this test was not valid<sup>24,25</sup>. The product of the exact probabilities of the observed or more extreme results in each volume was taken, and the probability of obtaining the observed product or a smaller one, out of the 720 possible products, was calculated using a computer (when the observed number of extinctions in each volume was kept, there were 12, 6, 5 and 2 possible probabilities in 4-, 12-, 50- and 300-l experiments, respectively). In the present case, the observed *P*-values in each volume are 0.4398 (4 l), 0.1889 (12 l), 0.00996 (50 l) and 0.8182 (300 l), and the probability of obtaining the product 0.0006768 or smaller is 0.004839 (all probabilities are one-tailed).

year in the larger volumes showed that there were significantly more extinctions in three-species experiments (13 of 45 populations) than in two-species experiments (8 of 64 populations) ( $P=0.00484$ ; Table 2). Of the 21 extinctions observed overall, the large species *D. magna* went extinct fewer times than the smaller *D. pulex* and *D. longispina* (2, 9 and 10 extinctions respectively; Table 2; pooled data, *G*-test:  $G=6.97$ , 2 degrees of freedom,  $P<0.05$ ).

Detailed analyses of population dynamics and reproduction demonstrated that the three species used common food resources, and that this led to severely depressed food levels, strongly reduced reproductive rates, and intense interspecific competition during substantial parts of each year<sup>11</sup>. Because predators were excluded by insect nets, and the experimental vessels never dried up, the observed extinctions most probably were attributable to interspecific competition. The populations that subsequently went extinct generally had lower mean densities than persisting ones, but the coefficients of variation in density did not differ between extinct and persisting populations (Table 1). Thus small population sizes in conjunction with intense interspecific competition apparently made populations in these experiments vulnerable to extinction.

All three species have not been observed together in the same pool either in natural rockpools in the three Swedish areas<sup>11</sup>, or in southwestern Finland<sup>12,15</sup> (Tables 3 and 4). Extinction rates in two-species pools in all the four areas were higher than in one-species pools; on average 18 and 11% of the populations went extinct per year, respectively (Table 3; *n*-weighted averages)<sup>11,15</sup>. In natural rockpools, there were no significant consistent differences in extinction rates between the three species (Table 4).

Thus, interspecific competition seems to influence the distribution and metapopulation dynamics of *Daphnia* species in the field<sup>11,14</sup>. In particular, it may contribute to the scarcity of three-species pools by reducing the longevity of three-species combinations. Several observations, however, suggest that interspecific competition is only one of several factors causing natural extinctions in rockpools. First, no extinctions occurred in the larger (50- and 300-litre) two-species experiments (Tables 1 and 2). Second, many population turnovers in natural rockpools

could be attributed to variation in abiotic factors such as salinity<sup>11</sup>. Third, predators such as newts, fish and water bugs, if present, can easily drive *Daphnia* populations to extinction<sup>11,18,19</sup>.

The results presented here indicate that interspecific competition may limit species richness in the rockpool metapopulation system. Two *Daphnia* species seem to be able to coexist in single pools for extended periods of time despite no, or little, possibility for niche differentiation (ref. 11, and personal observations). The three species coexist regionally, but not all together in single pools, in several areas in Scandinavia<sup>11-15</sup>, a finding that is

TABLE 3 Extinction rates in natural rockpools with one- and two *Daphnia* species in four areas of Scandinavia

| Area       | Extinction rate (probability of extinction per population per year) $\pm$ s.d. |                                      |
|------------|--------------------------------------------------------------------------------|--------------------------------------|
|            | One species present                                                            | Two species present                  |
| Flatholmen | 0.13 ( $\pm 0.037$ ) ( <i>n</i> =82)                                           | 0.15 ( $\pm 0.046$ ) ( <i>n</i> =58) |
| Mönster    | 0.12 ( $\pm 0.038$ ) ( <i>n</i> =74)                                           | 0.42 ( $\pm 0.14$ ) ( <i>n</i> =12)  |
| Ängskär    | 0.097 ( $\pm 0.025$ ) ( <i>n</i> =143)                                         | 0.17 ( $\pm 0.051$ ) ( <i>n</i> =54) |
| Tvärminne  | 0.11 ( $\pm 0.028$ ) ( <i>n</i> =123)                                          | 0.16 ( $\pm 0.052$ ) ( <i>n</i> =50) |

*n*, Total number of possible extinction events during the study period. There were significantly more extinctions in rockpools with two species present than in one-species pools (Fisher's combined probability test<sup>23</sup> using probabilities from *G*-tests for each area separately:  $P=18.25$ , eight degrees of freedom,  $P<0.02$ ). The areas are Flatholmen island and Mönster peninsula on the Swedish west coast<sup>11</sup>, Ängskär islands in the Stockholm archipelago on the Swedish east coast<sup>11</sup>, and islands near Tvärminne in south-western Finland<sup>15</sup>. Extinction rates on Mönster and Ängskär were calculated using data on species occurrences in rockpools during 1982–86, and on Flatholmen using data from 1982–84 and 1986–87. An extinction was judged to have occurred if a species present in one year was absent from the samples in two successive years. Extinction rates were calculated as the sum of the number of observed extinctions in populations present in each of the years 1982, 1983 and 1984, divided by the total number of possible extinctions during these years. See also ref. 11. In the Tvärminne area, extinction rates were calculated as the number of populations present in 1983 that were extinct by 1985, divided by the number of populations present in 1983 (Tables 2 and 6 in ref. 15 were used).

TABLE 4 Number of extinctions of *Daphnia* species in natural rockpools in three areas in Sweden

| Area       | M         | P        | L        | Number of extinctions of <i>Daphnia</i> in different species combinations |                          |                        |           |
|------------|-----------|----------|----------|---------------------------------------------------------------------------|--------------------------|------------------------|-----------|
|            |           |          |          | M + P                                                                     | M + L                    | P + L                  | M + P + L |
| Flatholmen | 12 (n=60) | 0 (n=5)  | 3 (n=17) | 0 (n=0)                                                                   | M 7 (n=29)<br>L 5 (n=29) | 0 (n=0)                | 0 (n=0)   |
| Mönster    | 6 (n=61)  | 3 (n=13) | —        | M 2 (n=6)<br>P 3 (n=6)                                                    | —                        | —                      | —         |
| Ängskär    | 6 (n=46)  | 4 (n=75) | 4 (n=22) | M 1 (n=16)<br>P 3 (n=16)                                                  | M 2 (n=10)<br>L 2 (n=10) | P 0 (n=1)<br>L 1 (n=1) | 0 (n=0)   |

n, Number of possible extinction events in each species. *Daphnia magna* (M), *D. pulex* (P) and *D. longispina* (L). No *D. longispina* were found in the Mönster area. There were no significant differences in extinction rates between the three species (G-tests,  $P > 0.05$ ).

consistent with immigration-extinction models for coexistence of similar competitors in patchy habitats<sup>14,16,20</sup>. The field experiments demonstrated, however, that extinction rates increased with an increasing number of species initially present; this indicates that regional coexistence of a large number of cladoceran species in this system is unlikely.

It has been debated whether interspecific competition is an important factor determining species distributions and limiting species richness in natural communities<sup>1-7,21,22</sup>. The combination of experimental and observational evidence presented here shows that interspecific competition can be important in these respects, and that it may be essential to incorporate the process in studies of metapopulation systems. □

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## Noradrenaline and serotonin selectively modulate thalamic burst firing by enhancing a hyperpolarization-activated cation current

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NEURONS in many regions of the mammalian nervous system generate action potentials in two distinct modes: rhythmic oscillations in which spikes cluster together in a cyclical manner, and single spike firing in which action potentials occur relatively independently of one another<sup>1,2</sup>. Which mode of action potential generation a neuron displays often varies with the behavioural state of the animal<sup>2,3</sup>. For example, the shift from slow-wave sleep to waking and attentiveness is associated with a change in thalamic neurons from rhythmic burst firing to repetitive single spike activity, and a greatly increased responsiveness to excitatory synaptic inputs<sup>1-3</sup>. This marked change in firing pattern and excitability is controlled in part by ascending noradrenergic and serotonergic

inputs from the brainstem<sup>4-11</sup>, although the cellular mechanisms of this effect have remained largely unknown. Here we report that noradrenaline and serotonin enhance a mixed  $\text{Na}^+/\text{K}^+$  current which is activated by hyperpolarization ( $I_h$ ) and that this enhancement may be mediated by increases in intracellular concentration of cyclic AMP. This novel action of noradrenaline and serotonin reduces the ability of thalamic neurons to generate rhythmic burst firing and promotes a state of excitability that is conducive to the thalamocortical synaptic processing associated with cognition.

Application of noradrenaline (NA; 0.5 mM) or serotonin (5-HT; 0.3 mM) to guinea-pig ( $n = 125$ ) or cat ( $n = 6$ ) dorsal lateral and medial geniculate neurons at resting membrane potential ( $-60$  to  $-68$  mV), resulted in a small depolarization associated with an increase in apparent membrane conductance (Fig. 1a, b). The current-versus-voltage ( $I-V$ ) relationships show that both the NA- and 5-HT effects were highly voltage-dependent and appeared as an inward current at membrane potentials negative to  $\sim -60$  mV (Fig. 1c, d). The membrane potential at which the response first became apparent was similar for NA ( $-65 \pm 7$  mV, mean  $\pm$  s.d.,  $n = 18$ ) and 5-HT ( $-69 \pm 6$  mV,  $n = 7$ ). This effect of NA and 5-HT was a direct postsynaptic response because it persisted after blocking synaptic transmission by exposing the tissue slices to a low- $\text{Ca}^{2+}$  (0.5 mM)- and high  $\text{Mn}^{2+}$  (4 mM)-containing medium ( $n = 3$ ). In contrast to the voltage-dependent responses to NA and 5-HT (Fig. 1c, d), increases in membrane  $\text{Cl}^-$  conductance, elicited by the GABA<sub>A</sub> ( $\gamma$ -aminobutyric acid) agonist muscimol ( $n = 11$ ), and  $\text{K}^+$  conductance, elicited by the GABA<sub>B</sub> agonist baclofen ( $n = 7$ )<sup>12</sup>, displayed reversal potentials and were less voltage dependent (Fig. 1e, f).

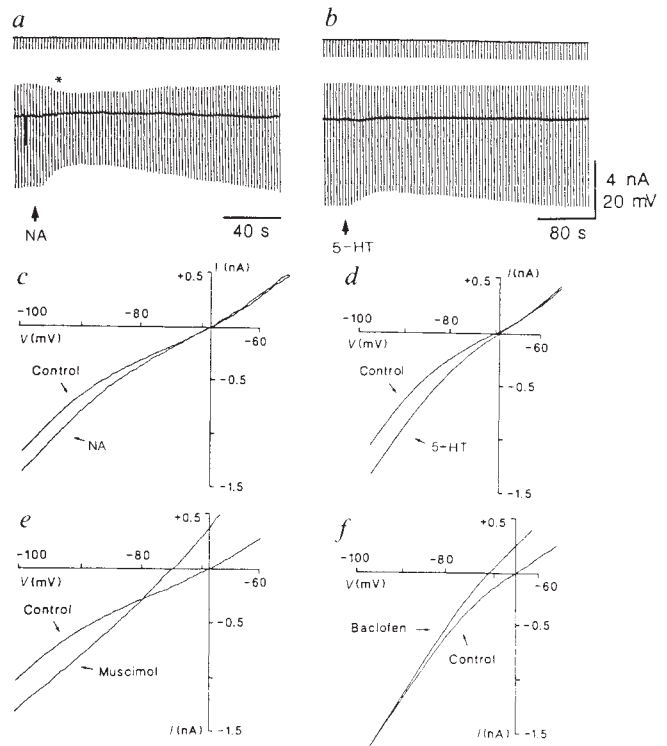
The NA-induced response appeared to be mediated by  $\beta$ -adrenergic receptors because prazosin (2  $\mu\text{M}$ ) and yohimbine (1  $\mu\text{M}$ ) were routinely added to the perfusion medium to block

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FIG. 1 Responses of thalamic neurons to NA and 5-HT. *a*, Application of NA to a guinea pig dorsal lateral-geniculate neuron during the injection of hyperpolarizing constant current pulses (top trace) results in a small depolarization and a large increase in apparent membrane conductance (bottom trace). Upward going lines in this and all subsequent voltage traces represent rebound  $\text{Ca}^{2+}$  spikes (indicated by asterisk; spikes are truncated by slow frequency response of chart recorder). *b*, Application of 5-HT to a cat medial geniculate neuron has a similar effect. *c*, *d*, The  $I-V$  relationships obtained in voltage clamp before and after application of NA and 5-HT show a progressive increase in inward current at membrane potentials negative to about  $-60$  mV. *e*, In contrast to NA and 5-HT responses, increasing membrane  $\text{Cl}^-$  conductance by activating  $\text{GABA}_A$  receptors with muscimol ( $100 \mu\text{M}$ ) shows no such voltage sensitivity. *f*, Increasing membrane  $\text{K}^+$  conductance by activating  $\text{GABA}_B$  receptors with baclofen ( $100 \mu\text{M}$ ) results in an outward current that reverses at about  $-105$  mV. Data in *c-f* are obtained from the same guinea pig dorsal lateral-geniculate neuron. The small depolarizations associated with responses to NA and 5-HT in *a*, *b* were smaller than usual.

**METHODS.** Guinea pigs or cats (two) were deeply anaesthetized with sodium pentobarbital and killed by decapitation, as described previously<sup>28-30</sup>. The medial and lateral geniculate nuclei were rapidly dissected free and were sectioned coronally on a vibratome as  $400\text{-}\mu\text{m}$  slices. Slices were maintained in an interface chamber at  $36 \pm 1^\circ\text{C}$  and perfused with a solution containing (mM): NaCl (126), KCl (2.5),  $\text{MgSO}_4$  (2),  $\text{NaHCO}_3$  (26),  $\text{NaH}_2\text{PO}_4$  (1.25),  $\text{CaCl}_2$  (2), glucose (10); saturated with 95%  $\text{O}_2$ , 5%  $\text{CO}_2$  to final pH 7.4. Changing extracellular ionic concentrations was achieved by the following substitutions: choline chloride for NaCl; KCl for NaCl; and sodium isethionate for NaCl, potassium acetate for KCl. When  $\text{Mn}^{2+}$  was added to the bathing medium, sulphate and phosphate were omitted to prevent precipitation. Only neurons having stable membrane potentials negative to  $-55$  mV and overshooting action potentials were included for analysis. Unless stated otherwise, neuroactive substances were dissolved in the bathing medium and applied in volumes of 5–20  $\mu\text{l}$  locally to the surface of the slice near the recording site through a broken intracellular micropipette (2–5- $\mu\text{m}$  tip diameter) by pulses of pressure. Intracellular recording microelectrodes were filled with 4 M potassium acetate and had a final resistance of 25–50  $\text{M}\Omega$ . Single electrode voltage clamp was achieved with an Axoclamp-2A amplifier (Axon Instruments) coupled to an IBM AT computer operating pClamp software (Axon Instruments). Headstage output was continuously monitored and sampling frequencies were between 3.5 and 5.5 KHz.



Amplifier gain was typically  $\sim 1 \text{ nA mV}^{-1}$ .  $I-V$  relationships were obtained by applying a hyperpolarizing voltage ramp from  $\sim -50$  to  $-100$  mV. Thalamic neurons respond to NA with large depolarizations through a decrease in  $\text{K}^+$  conductance mediated by  $\alpha_1$ -receptors<sup>29</sup>. To isolate the effects of NA through  $\beta$ -receptors for the present study, prazosin ( $2 \mu\text{M}$ ) and yohimbine ( $1 \mu\text{M}$ ) were routinely added to the bathing medium. Responses to 5-HT and isoprenaline were identical in either normal or prazosin-yohimbine containing medium.

$\alpha$ -receptors, and NA-induced responses were mimicked by the  $\beta$ -adrenergic-specific agonist isoprenaline ( $50\text{--}250 \mu\text{M}$ ,  $n = 12$ ). Application of the  $\beta$ -adrenergic antagonists propranolol (local application,  $100 \mu\text{M}$  in micropipette,  $n = 2$ ) and atenolol ( $15 \mu\text{M}$  in the bath,  $n = 2$ ) reversibly blocked responses to NA but not to 5-HT. By contrast, local ( $10\text{--}100 \mu\text{M}$ ,  $n = 4$ ) or bath ( $1\text{--}5 \mu\text{M}$ ,  $n = 4$ ) application of the 5-HT<sub>1</sub> and 5-HT<sub>2</sub> antagonist methysergide blocked the response to serotonin without affecting the response to NA. The 5-HT<sub>1A</sub> agonist ipsapirone ( $0.2 \text{ mM}$ ), the partial agonist 8-OHDPAT (8-hydroxy-dipropylaminotetralin) ( $0.1\text{--}1 \text{ mM}$ ,  $n = 4$ ), the 5-HT<sub>2</sub> antagonist ritanserin ( $5 \mu\text{M}$  in the bath,  $n = 4$ ), all had no effect on dorsal lateral geniculate neurons or on the response of these neurons to 5-HT. This

indicates that the 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> receptor subtypes are not involved in the response to 5-HT. This finding is in agreement with the non-5-HT<sub>1A</sub>, non-5-HT<sub>2</sub> pharmacological profile of serotonin binding-sites in the medial and lateral geniculate nuclei<sup>13,14</sup>.

The marked similarity between the NA- and 5-HT responses suggests that they may have been affecting the same current. Indeed, we found that a maximal application of 5-HT greatly reduced or blocked the response to NA in voltage clamp ( $n = 4$ ), a phenomenon known as non-additivity, indicative of convergence of neurotransmitter action<sup>15</sup>.

Because the NA- and 5-HT-induced responses were strongly voltage dependent, we hypothesized that NA and 5-HT could

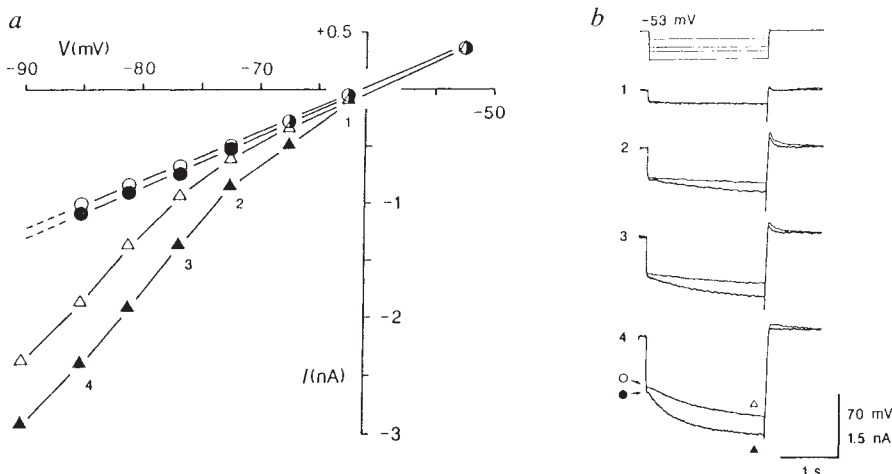


FIG. 2 Noradrenaline enhances a current activated by hyperpolarization. *a*, Graph of the instantaneous (circles) and steady-state (triangles) currents in response to a hyperpolarizing voltage step before (open symbols; control) and after (filled symbols) application of NA. Original traces are shown in *b*. Application of NA results in a marked enhancement of the inward current activated by hyperpolarization, with only a small increase in instantaneous conductance.



have been enhancing a current activated by hyperpolarization. Neurons in other brain regions, and cells in the heart, possess inward currents that are activated by hyperpolarization and have been variously termed  $I_Q$ ,  $I_f$ ,  $I_{AR}$ ,  $I_h$  and  $I_{Cl(v)}$ <sup>16-22</sup>. To test for the presence of such currents in thalamic neurons, we performed incrementing voltage steps from a holding potential of  $-50$  mV to between  $-60$  and  $-100$  mV. Hyperpolarization negative to  $-60$  mV activated a slowly developing inward current, which we term  $I_h$ . The amplitude and rate of activation of  $I_h$  increased with membrane hyperpolarization (Fig. 2). The time course of activation of  $I_h$  was well fitted (coefficient of correlation,  $r > 0.99$ ) to a single exponential function with a time constant of between  $0.2$  ( $-100$  mV) and  $2.0$  ( $-70$  mV) seconds. Application of NA or 5-HT resulted in a substantial enhancement of  $I_h$ , with little, if any, change in instantaneous membrane conductance (Fig. 2,  $n = 11$ ). Interestingly, the activation time course of  $I_h$  was significantly faster after application of NA or 5-HT (time constant =  $1,092 \pm 262$  ms pre-application;  $712 \pm 166$  ms post-application;  $t = 4.8$   $P < 0.01$ ; voltage step from  $-50$  to  $-80$  mV;  $n = 5$ ), as has been reported for noradrenergic enhancement of  $I_f$  in the heart<sup>23-25</sup>.

Stimulation of  $\beta$ -adrenergic receptors in many regions of the nervous system leads to increases in the intracellular concentration of cAMP (ref. 26). In the heart, it is this increase in intracellular cAMP that is thought to couple  $\beta$ -adrenergic receptors to increases in the hyperpolarization activated 'pacemaker' current  $I_f$  (ref. 25). In thalamic neurons we found that local application of the membrane-permeable cAMP analogue 8-bromo-cAMP ( $500 \mu\text{M}$ ,  $n = 5$ ), the adenylyl cyclase stimulant forskolin ( $25 \mu\text{M}$ ,  $n = 4$ ), or the phosphodiesterase inhibitor 3-isobutyl-1-methyl-xanthine (IBMX,  $300 \mu\text{M}$ ,  $n = 3$ ) all resulted in a marked enhancement of  $I_h$  with little or no change in instantaneous 'leak' conductance (data not shown). Local application of the forskolin analogue 1,9-dideoxy-forskolin ( $50$ – $100 \mu\text{M}$ ,  $n = 4$ ), which does not activate adenylyl cyclase, had no effect. These results suggest that the  $\beta$ -adrenergic and/or serotonergic enhancement of  $I_h$  may be mediated by an increase in intracellular concentration of cAMP.

The ionic nature underlying  $I_h$  and the NA- and 5-HT-induced response was investigated by changing the concentration gradient of  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  across the neuronal membrane (Fig. 3). Reducing the extracellular concentration of  $\text{Na}^+$  ( $[\text{Na}^+]_o$ ) from  $153$  mM to  $26$  mM resulted in a parallel shift on the voltage axis of the  $I$ - $V$  curve for  $I_h$  and the NA response by  $-14$  mV ( $\pm 3$  mV,  $n = 4$ ) (Fig. 3a). Similarly, shifting  $[\text{Na}^+]_o$  from  $26$  mM to  $153$  mM shifted the  $I$ - $V$  curve by  $+19$  mV ( $\pm 7$  mV,  $n = 3$ ). Increasing  $[\text{K}^+]_o$  from  $2.5$  to  $7.5$  mM shifted  $I_h$  and the NA-induced response by  $+11$  mV ( $\pm 1$  mV,  $n = 6$ ) (Fig.

3b). Returning  $[\text{K}^+]_o$  to normal reinstated the original response in one cell. These observed changes correspond to a shift of  $\sim 21$  and  $25$  mV per tenfold change in  $[\text{Na}^+]_o$  and  $[\text{K}^+]_o$ , respectively.

Reducing  $[\text{Cl}^-]_o$  from  $132.5$  to  $4$  mM did not affect the response to NA (Fig. 3c,  $n = 5$ ), but did result in a large shift (up to  $+70$  mV) in the reversal potential of responses to the GABA<sub>A</sub> agonist muscimol (Fig. 3c). Similarly, intracellular injection of  $\text{NO}_3^-$ , which readily passes through  $\text{Cl}^-$  channels but does not support outward  $\text{Cl}^-$  transport<sup>27</sup>, resulted in large

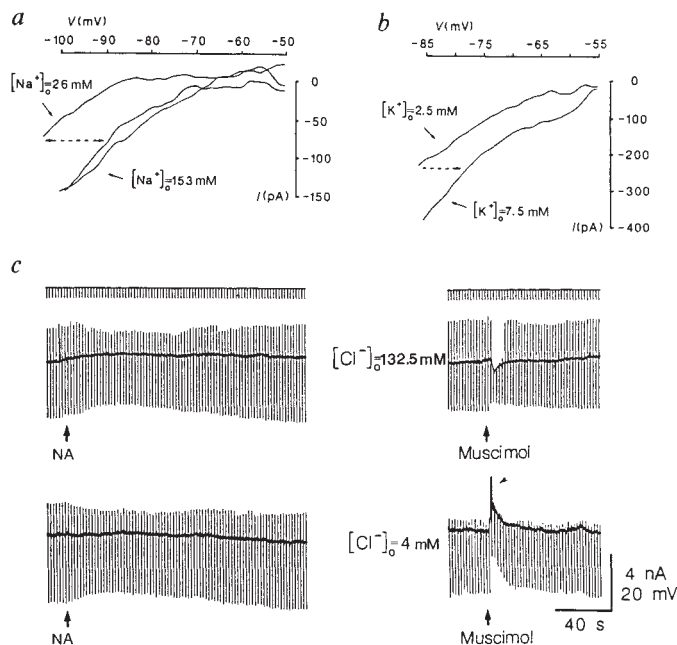
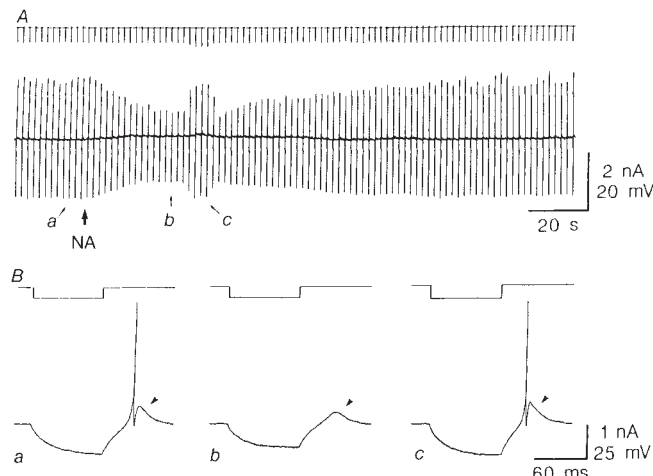


FIG. 3 Effects of changing extracellular  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  concentrations on the response to NA. *a*, Plot of the current activated by NA versus membrane potential in normal  $[\text{Na}^+]_o$  ( $153$  mM), after washing in low  $[\text{Na}^+]_o$  ( $26$  mM), and recovery back to normal  $[\text{Na}^+]_o$ . The NA-response  $I$ - $V$  curve is found to shift by  $\sim 16$  mV. *b*, Changing  $[\text{K}^+]_o$  in another neuron from  $2.5$  to  $7.5$  mM shifts the curve by  $\sim +12$  mV. The NA-response  $I$ - $V$  curves were obtained by subtracting the  $I$ - $V$  plots with NA from those obtained before NA addition. *c*, Reducing  $[\text{Cl}^-]_o$  from  $132.5$  to  $4$  mM shifts the response to the GABA<sub>A</sub> agonist muscimol from a small hyperpolarization to a large depolarization, which elicits action potentials (arrow head). By contrast, reducing  $[\text{Cl}^-]_o$  has no effect on the response to NA.

FIG. 4 Effect of NA on the response of a lateral geniculate neuron to hyperpolarizing current pulses. Intracellular injection of hyperpolarizing current pulses typically results in a rebound  $\text{Ca}^{2+}$ -mediated spike<sup>32,33</sup> (*B*, arrow heads) that can activate  $\text{Na}^+/\text{K}^+$ -mediated action potentials. Application of NA substantially decreases the hyperpolarizing response and subsequently reduces the amplitude of the rebound  $\text{Ca}^{2+}$ -mediated spike (*B*, compare *a* and *b*). Increasing the amplitude of the current pulse so that the hyperpolarization matches that before NA addition reinstates the full  $\text{Ca}^{2+}$ -mediated spike (*B*, *c*). Traces expanded in *B* are as indicated in *A*. The ability of NA to reduce all components of the hyperpolarizing pulse results from an increase in the amount of  $I_h$  active at resting membrane potential as well as the amount that is activated by the hyperpolarizing pulse (data not shown).



depolarizing responses to muscimol, but normal responses to NA ( $n=6$ , not shown).

These results demonstrate that NA and 5-HT enhance a hyperpolarization-activated current ( $I_h$ ) that is carried by  $\text{Na}^+$  and  $\text{K}^+$  ions. In further support of this, we found that both  $I_h$  and the response to NA and 5-HT were blocked by introducing  $\text{Cs}^+$  (2 mM) but not  $\text{Ba}^{2+}$  (0.5 mM) into the bathing medium ( $n=9$ ).

Rhythmic burst firing in thalamic relay neurons during periods of electroencephalographic (EEG) synchronization (such as slow-wave sleep) results from the generation of low threshold  $\text{Ca}^{2+}$  spikes, which appear as rebound responses arising from rhythmic inhibitory postsynaptic potentials (i.p.s.p.s) from the nucleus reticularis<sup>1,2</sup>. To test a possible effect of NA or 5-HT on rhythmic burst firing, we injected short duration (40–80 ms, 0.5–4.0 Hz) hyperpolarizing current pulses to mimic rhythmic i.p.s.p.s. Application of NA or 5-HT substantially decreased both the voltage deviation caused by the hyperpolarizing pulse as well as the rebound  $\text{Ca}^{2+}$  spike (Fig. 4). The latter was a secondary effect, as increasing the pulse size reinstated the full response (Fig. 4). By contrast, NA had only very weak facilitatory effects on the response of these same cells to depolarizing current pulses and no consistent effect on the firing rate of neurons that were manually depolarized above single-spike firing threshold (about  $-55$  mV, data not shown).

The results of this study reveal a potent and novel action for NA and 5-HT in the central nervous system. In the thalamus, the NA- and 5-HT-induced increase in  $I_h$  results in a selective dampening of neuronal responsiveness to large hyperpolarizing inputs, with little effect on phasic or tonic depolarizations. Together with the other actions of acetylcholine and NA, this effect may be responsible for the marked increase in the efficacy of transfer of information through the thalamus during periods of increased arousal and attentiveness<sup>28–31</sup>. The coexistence of  $I_h$  or analogous currents, and a serotonergic or noradrenergic projection at all levels of the nervous system, implies that the present findings may have widespread implications for the neurotransmitter control of neuronal excitability<sup>16–22</sup>. □

## Chloride conductance regulated by cyclic AMP-dependent protein kinase in cardiac myocytes

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In heart cells, cyclic AMP-dependent protein kinase (PKA) regulates calcium<sup>1–4</sup> and potassium-ion current<sup>1,2,5,6</sup> by phosphorylating the ion channels or closely associated regulatory proteins. We report here that isoprenaline induced large chloride-ion currents in voltage-clamped, internally-dialysed myocytes from guinea-pig ventricles. The  $\text{Cl}^-$  current could be activated by intracellular dialysis with cAMP or the catalytic subunit of PKA, indicating regulation by phosphorylation. In approximately symmetrical solutions of high  $\text{Cl}^-$  concentration, the macroscopic cardiac  $\text{Cl}^-$  current showed little rectification, unlike the single-channel current in PKA-regulated  $\text{Cl}^-$  channels of airway epithelial cells<sup>7</sup>. But, like epithelial  $\text{Cl}^-$ -channel currents, the cardiac  $\text{Cl}^-$  current was sensitive to the distillbene, 4,4'-dinitrostilbene-2,2'-disulphonic acid (DNDS)<sup>8</sup>. In the absence of kinase activation, cardiac sarcolemmal  $\text{Cl}^-$  conductance was negligible. During  $\beta$ -adrenergic stimulation of the heart, this novel  $\text{Cl}^-$  conductance should accelerate action-potential repolarization and so protect impulse propagation in the face of the possibly arrhythmogenic increases in heart rate and in calcium entry into the cells.

Application of 100 nM isoprenaline to a myocyte bathed in 146 mM  $\text{Cl}^-$  solution, and equilibrated with  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$ -free pipette solution containing 135 mM  $\text{Cl}^-$ , caused little change in whole-cell current at the 0 mV holding potential, which was chosen to inactivate  $\text{Na}^+$  and  $\text{Ca}^{2+}$  channels (Fig. 1A). Voltage pulses of 80 ms to membrane potentials between  $-100$  mV and  $+100$  mV revealed, however, large isoprenaline-activated currents (Fig. 1B,  $b-a$ ), whose amplitude varied approximately linearly with voltage (Fig. 1D), and which reversed near 0 mV, close to the  $\text{Cl}^-$  equilibrium potential ( $E_{\text{Cl}}$ , estimated to be  $-2$  mV under these conditions). That the isoprenaline-induced current was indeed an anion current carried by  $\text{Cl}^-$ , and not a cation current carried, for example, by extracellular  $\text{Na}^+$  and intracellular  $\text{Cs}^+$  through a non-specific pathway, is shown by the virtual abolition of the inward current at negative potentials (Fig. 1B, C, D), and by the concomitant appearance of an isoprenaline-induced outward shift of the holding current at 0 mV (Fig. 1A), after exchange of the  $\text{Cl}^-$ -containing solution within the pipette for a  $\text{Cl}^-$ -free solution, containing instead 90 mM aspartate and 20 mM methanesulphonate.

Further evidence that the isoprenaline-activated current was carried by  $\text{Cl}^-$  is presented in Fig. 2A, which shows that, at positive potentials, the large outward current elicited by isoprenaline in a cell bathed in 146 mM  $\text{Cl}^-$  solution, was virtually absent after all but 1 mM extracellular  $\text{Cl}^-$  was replaced by isethionate ions. Because the intracellular  $\text{Cl}^-$  concentration ( $[\text{Cl}^-]_i$ ) was kept constant at 20 mM, this drastic reduction of extracellular  $\text{Cl}^-$  concentration ( $[\text{Cl}^-]_o$ ) caused the reversal potential for the isoprenaline-induced current (and  $E_{\text{Cl}}$ ) to shift from a negative potential to a positive potential (Fig. 2Ab). This, in turn, caused the isoprenaline-induced shift of the holding current at 0 mV to reverse from outward to inward (Fig. 2Aa). The record of whole-cell current in Fig. 2Ba shows that

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the pronounced outward current activated by isoprenaline at 146 mM  $[Cl^-]_o$  could be abolished by subsequent application of 0.1 mM DNDS, one of the disulphonic stilbene derivatives which are potent blockers of anion fluxes in a variety of cell types<sup>8-11</sup>. The whole-cell current-voltage ( $I-V$ ) relationships in Fig. 2Bb demonstrate that, over the entire voltage range examined, membrane current in the combined presence of isoprenaline and DNDS was almost the same as that in the absence of both.

Isoprenaline (100 nM) activated large steady  $Cl^-$  currents ( $>0.5$  pA pF<sup>-1</sup> at +100 mV) in 33 of the 42 cells tested at 146 mM  $[Cl^-]_o$ . The lack of any change in holding current (at 0 mV) on removing either intracellular  $Cl^-$  (Fig. 1A) or extracellular  $Cl^-$  (Fig. 2Aa) in the absence of isoprenaline, however, and the identity of whole-cell  $I-V$  relationships with and without internal  $Cl^-$  (Fig. 1C) or external  $Cl^-$  (not illustrated), indicates that membrane  $Cl^-$  conductance is negligible unless  $\beta$ -adrenoceptors are activated.

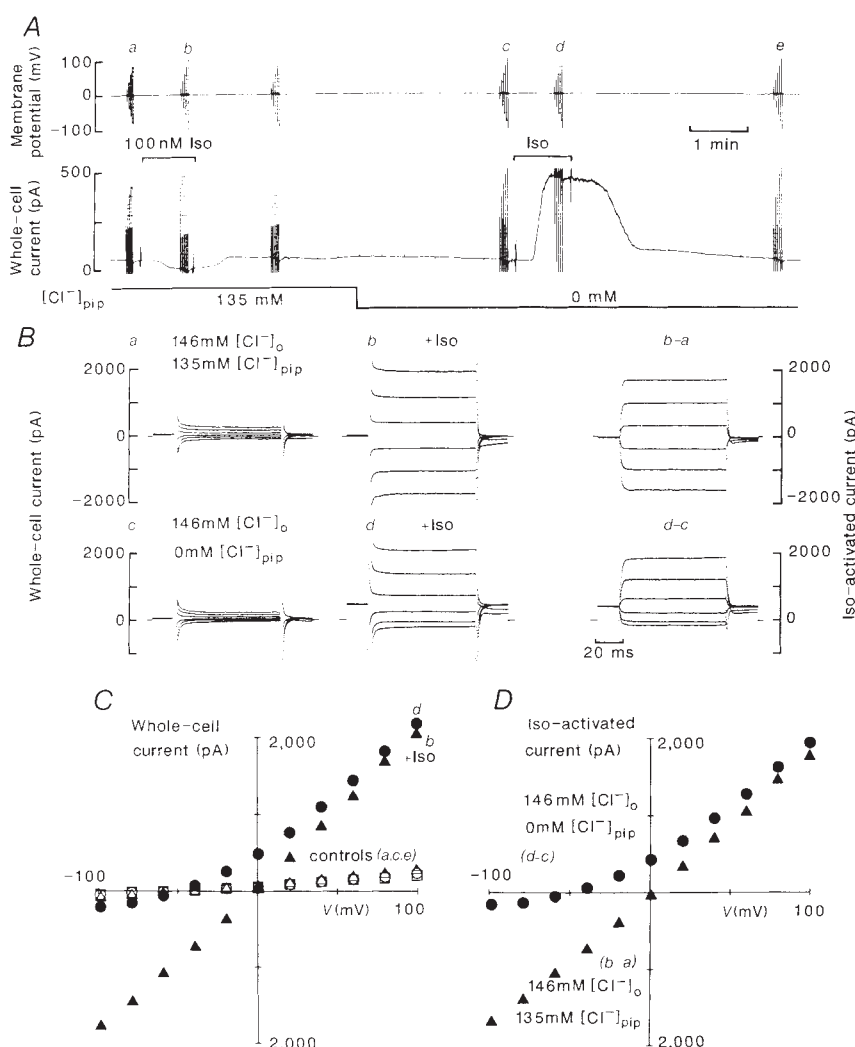
Like the unitary  $Cl^-$ -channel currents in airway epithelial cells<sup>12-15</sup> and lymphocytes<sup>16</sup>, these  $Cl^-$  currents elicited by isoprenaline in myocytes seem to be regulated by PKA. Thus, large outward currents, with voltage dependence similar to those activated by isoprenaline (Fig. 3Ab, Bb), were seen if myocytes

exposed to 146 mM  $[Cl^-]_o$  were internally dialysed with either cAMP (Fig. 3A) or the catalytic subunit of PKA (Fig. 3B). Furthermore, the strong outward current at positive potentials induced by putting cAMP in the pipette was diminished, in another cell, by ~80% with 0.01 mM external DNDS, and by >90% with 0.1 mM DNDS (not illustrated), and three-quarters of that activated by the PKA subunit was abolished on lowering  $[Cl^-]_o$  from 146 mM to 1 mM (Fig. 3Bb). With the wide-tipped, low resistance (1–5 M $\Omega$ ) pipettes used in these experiments, the  $Cl^-$  conductance was usually activated within seconds of switching to a pipette solution containing 1 mM cAMP (for example, see Fig. 3Aa), but took more than 15 min to appear after introducing the PKA catalytic subunit to the pipette tip in the experiment of Fig. 3Ba. Although small molecules like cAMP diffuse readily into the cell from the pipette<sup>1,17</sup>, the relatively large protein subunit (relative molecular mass  $M_r$  of 40,000) can be expected to take many minutes to half equilibrate with the cell interior<sup>1</sup>.

The cAMP-dependent  $Cl^-$  currents described here do not seem to require intracellular  $Ca^{2+}$  (refs 7, 13, 18), because  $Ca^{2+}$  currents were inactivated (Fig. 1B) by the 0 mV holding potential, and pipette solutions contained 10 mM (Figs 1, 2B and 3A) or 50 mM (Figs 2A and 3B) EGTA and no added  $Ca^{2+}$ .

FIG. 1 Voltage dependence and sensitivity to  $[Cl^-]_o$  of isoprenaline-induced current. A, Chart recording of membrane potential (upper trace) and whole-cell current (lower trace). Upper trace, the groups of vertical lines indicate acquisition of  $I-V$  data during 80-ms voltage pulses from the holding potential, 0 mV, to potentials between -100 and +100 mV. Lower trace, the bars mark exposure to 100 nM isoprenaline (Iso). The lower line indicates the change in pipette chloride concentration ( $[Cl^-]_{pip}$ ) from 135 to 0 mM (replaced by aspartate and methanesulphonate). Extracellular chloride concentration was constant at 146 mM. B, Superimposed records of whole-cell currents for pulses to +100, +60, +20, -60, and -100 mV, recorded in the absence (a, c) or presence (b, d) of isoprenaline, with either 135 mM  $[Cl^-]_{pip}$  (upper traces) or 0 mM  $[Cl^-]_{pip}$  (lower traces). Isoprenaline-activated currents ( $b-a$ ,  $d-c$ ) were obtained by subtracting currents recorded in the absence of isoprenaline from corresponding currents recorded during the exposure to isoprenaline. C, Whole-cell  $I-V$  relationships from the data in A and B, in the absence ( $\Delta$ ,  $\square$ ) or presence ( $\blacktriangle$ ,  $\bullet$ ) of isoprenaline, with either 135 mM  $Cl^-$ -containing ( $\Delta$ ,  $\blacktriangle$ ) or  $Cl^-$ -free ( $\square$ ,  $\bullet$ ) pipette solution: steady-state current levels were measured near the end of each pulse. D, Steady-state levels of isoprenaline-activated currents determined from data represented in B, plotted against membrane potential; 135 mM  $[Cl^-]_{pip}$  ( $\blacktriangle$ ,  $b-a$ ) or 0 mM  $[Cl^-]_{pip}$  ( $\bullet$ ,  $d-c$ ). Total cell capacitance = 138 pF; initial pipette resistance = 3.3 M $\Omega$ .

METHODS. Myocytes were isolated from guinea-pig ventricles with collagenase, and then incubated in a high  $K^+$ -concentration, low  $Ca^{2+}$ -concentration medium<sup>25</sup> before being superfused at 35 °C with Tyrode's solution containing (mM): NaCl (145), KCl (5.4),  $CaCl_2$  (1.8),  $MgCl_2$  (2.3), dextrose (5.5), HEPES/NaOH (5) (pH 7.4). Giga-ohm seals were obtained with wide-tipped, fire-polished, pipettes (resistance  $\approx 1-5$  M $\Omega$ ) filled with Tyrode's that was then exchanged<sup>26</sup>, before rupture of the cell membrane, for a  $Cl^-$ -free pipette solution containing (mM): CsOH (~145), aspartic acid (90),  $MgATP$  (1.0),  $Tris_2$ -creatine phosphate (5), tetraethylammonium-methanesulphonate (20), EGTA (10), HEPES (10) (pH 7.4). The  $[Cl^-]_{pip}$  was raised to 135 mM by adding  $Cl^-$  and omitting aspartic acid and methanesulphonate. Voltage-clamped<sup>27</sup> cells were then superfused with  $K^+$ -free, low  $Ca^{2+}$ -concentration (0.5 mM  $CaCl_2$ ) and low  $Mg^{2+}$ -concentration (0.5 mM  $MgSO_4$ ) Tyrode's solution. The holding potential was set to 0 mV to inactivate  $Na^+$  and  $Ca^{2+}$  channels (see Fig. 1B). Potassium-channel currents were minimized by omitting  $K^+$ , and including  $Cs^+$  and tetraethylammonium in pipette solutions;  $Na^+/K^+$  pump current was prevented by omitting  $Na^+$  from pipette solutions and  $K^+$  from external solutions, and  $Na^+/Ca^{2+}$  exchange current was prevented by the nominal absence of internal  $Na^+$  and  $Ca^{2+}$ . Reduced glutathione (0.1  $\mu$ M) and EDTA (20  $\mu$ M) were added to solutions containing isoprenaline to slow its oxidation. The osmolality of all solutions was  $\sim 300$  mosmol kg<sup>-1</sup>. Current and voltage signals

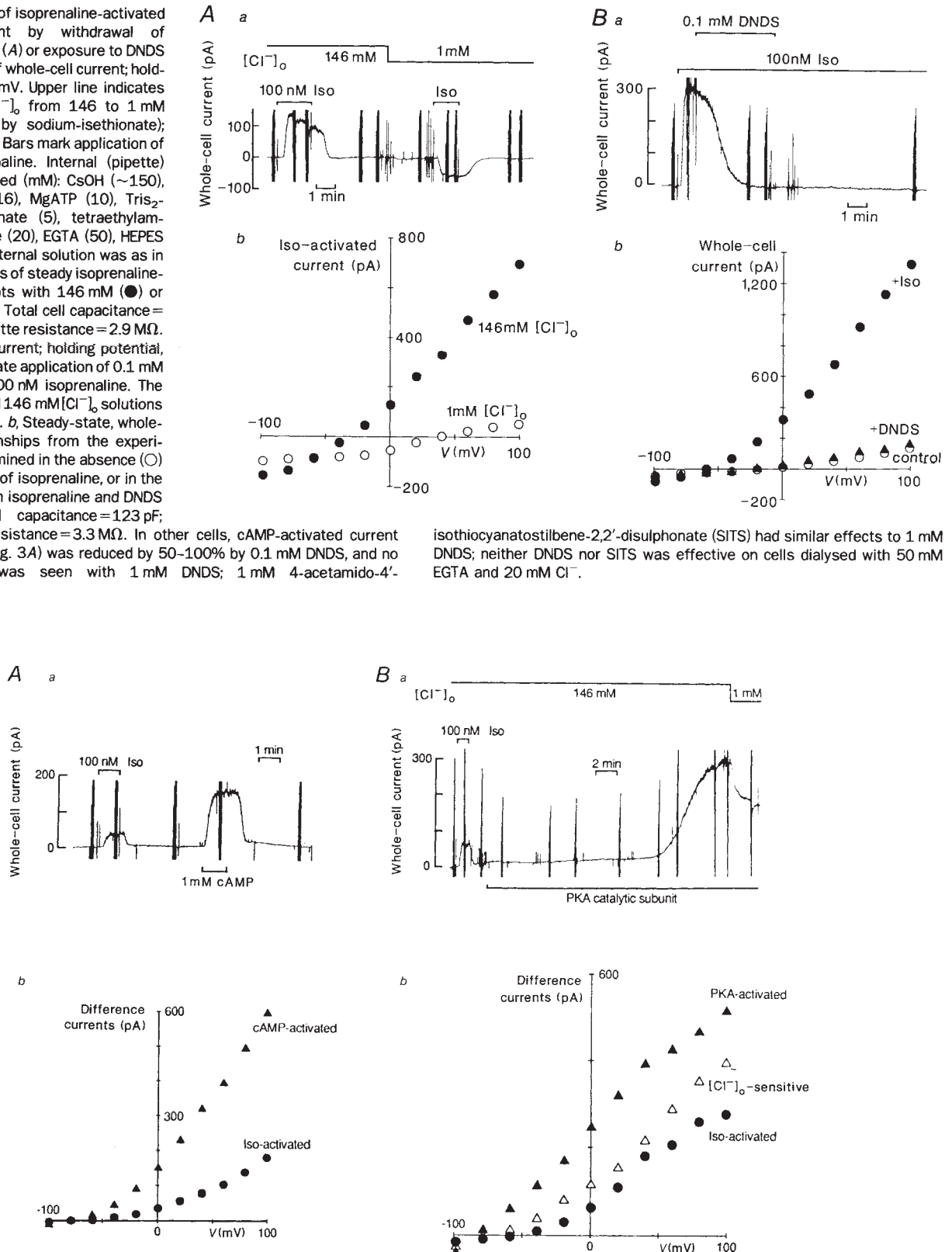


were low-pass filtered at 2 kHz (6-pole Bessel), digitized (12-bit resolution) on-line at 8 kHz, and stored in a microcomputer for analysis. To minimize voltage error due to a change of liquid-junction potential on changing solution composition, pipette and bath potentials were both measured by means of miniature KCl (3 M)-filled half cells.



**FIG. 2** Removal of isoprenaline-activated outward current by withdrawal of extracellular  $\text{Cl}^-$  (A) or exposure to DNDS (B). **Aa**, record of whole-cell current; holding potential, 0 mV. Upper line indicates reduction of  $[\text{Cl}^-]_o$  from 146 to 1 mM ( $\text{NaCl}$  replaced by sodium-isethionate);  $[\text{Cl}^-]_{pip} = 20$  mM. Bars mark application of 100 nM isoprenaline. Internal (pipette) solution contained (mM): CsOH (~150), aspartic acid (16), MgATP (10), Tris<sub>2</sub>-creatine phosphate (5), tetraethylammonium chloride (20), EGTA (50), HEPES (40) (pH 7.4). External solution was as in Fig. 1. **b**,  $I-V$  plots of steady isoprenaline-activated currents with 146 mM (●) or 1 mM (○)  $[\text{Cl}^-]_o$ . Total cell capacitance = 89 pF; initial pipette resistance = 2.9 MΩ. **Ba**, Whole-cell current; holding potential, 0 mV. Bars indicate application of 0.1 mM DNDS and/or 100 nM isoprenaline. The 0 mM  $[\text{Cl}^-]_{pip}$  and 146 mM  $[\text{Cl}^-]_o$  solutions were as in Fig. 1. **b**, Steady-state, whole-cell  $I-V$  relationships from the experiment in **a**, determined in the absence (○) or presence (●) of isoprenaline, or in the presence of both isoprenaline and DNDS (▲). Total cell capacitance = 123 pF; initial pipette resistance = 3.3 MΩ. In other cells, cAMP-activated current (compare with Fig. 3A) was reduced by 50–100% by 0.1 mM DNDS, and no further block was seen with 1 mM DNDS; 1 mM 4-acetamido-4'-

isothiocyanatostilbene-2,2'-disulphonate (SITS) had similar effects to 1 mM DNDS; neither DNDS nor SITS was effective on cells dialysed with 50 mM EGTA and 20 mM  $\text{Cl}^-$ .



**FIG. 3** Activation of  $\text{Cl}^-$  current by intracellular dialysis with either cAMP (A) or PKA catalytic subunit (B). **Aa**, Whole-cell current at 0 mV. Bars indicate exposure to isoprenaline or cell dialysis with 1 mM cAMP (in addition to 10  $\mu\text{M}$  IBMX to diminish phosphodiesterase activity). The  $[\text{Cl}^-]_{pip}$  was 0 mM. **b**, Steady-state  $I-V$  plots comparing isoprenaline-activated current (●) with cAMP-activated current (▲). Total cell capacitance = 150 pF; initial pipette resistance = 1.5 MΩ. **Ba**, Whole-cell current at 0 mV. The upper line marks a change of  $[\text{Cl}^-]_o$  from 146 to 1 mM; the short horizontal bar shows the exposure to isoprenaline; the lower bar indicates the period of intracellular

dialysis with a pipette solution containing 20 mM  $\text{Cl}^-$  (see Fig. 2A) and PKA catalytic subunit (0.3 mg ml<sup>-1</sup>, specific activity 18  $\mu\text{mol min}^{-1} \text{mg}^{-1}$ ). Both pipette solutions included 2 mg ml<sup>-1</sup> bovine serum albumin (BSA; Sigma, Fraction V). In the absence of PKA catalytic subunit, BSA had no effect on whole-cell current. The catalytic subunit of PKA was prepared from bovine heart as previously described<sup>28</sup>. **b**, Steady-state  $I-V$  plots comparing current activated by isoprenaline (●) or by PKA catalytic subunit (▲) with the  $[\text{Cl}^-]_o$ -sensitive component of PKA-activated current (△). Total cell capacitance = 98 pF; initial pipette resistance = 2.0 MΩ.

The lack of any  $\text{Cl}^-$ -sensitive component of the steady membrane current without kinase activation (Figs 1 and 2) helps to explain the previous uncertainty about the existence of a  $\text{Cl}^-$  current in cardiac cells<sup>9,19-21</sup>. But the absence of earlier reports of the large  $\text{Cl}^-$  currents activated by  $\beta$ -catecholamine as described here is more puzzling, given recent studies of regulation of other currents in cardiac cells through the  $\beta$ -adrenoceptor/cAMP pathway<sup>1,2,5,6,22,23</sup>. Most of these studies focused on relatively large voltage- and time-dependent  $\text{K}^+$  or  $\text{Ca}^{2+}$  currents, and so activation of the practically time-independent  $\text{Cl}^-$  current (Fig. 1B) was presumably either obscured by oppositely directed changes in a steady component of the  $\text{Ca}^{2+}$  channel current, or possibly ignored. Egan *et al.*<sup>22,23</sup> did report isoprenaline-induced inward shifts of current at large negative potentials, and outward shifts at large positive potentials, not unlike those described here, but they tentatively attributed them to enhanced  $\text{Na}^+$  and  $\text{K}^+$  currents, respectively, in part because the inward shift was diminished after replacing extracellular  $\text{Na}^+$  by tetramethylammonium ions. Curiously, we found that replacement of all external  $\text{Na}^+$  by 145 mM N-methyl-D-glucamine<sup>+</sup>, but not by  $\text{Cs}^+$ , nearly abolished isoprenaline-activated inward and outward current.

Nevertheless, we believe that the currents reported here reflect  $\text{Cl}^-$ -channel activity rather than electrogenic co-transport, because the currents could be large (up to  $15 \mu\text{A cm}^{-2}$  at +100 mV, assuming specific membrane capacitance =  $1 \mu\text{F cm}^{-2}$ ), they reversed near  $E_{\text{Cl}}$  at high  $[\text{Cl}^-]_o$  and  $[\text{Cl}^-]_i$  (Fig. 1C), and the holding-current shift was invariably accompanied by large-amplitude, low-frequency current noise (Figs 1-3). Analysis of this current noise and identification of corresponding unitary  $\text{Cl}^-$ -channel currents in myocytes should settle this question.

The relationship between these putative cardiac  $\text{Cl}^-$  channels and those from the membranes of airway epithelial cells, known to be activated by PKA-mediated phosphorylation, remains unclear. The macroscopic cardiac  $\text{Cl}^-$  currents were (1) absent unless the cAMP pathway was activated, (2) showed almost no rectification with roughly symmetrical chloride concentrations, (3) showed little evidence of voltage-dependent gating, and (4) were irreversibly inhibited by DNDS. In contrast, epithelial  $\text{Cl}^-$  channels, whose regulation through PKA is defective in cystic fibrosis<sup>12-15</sup>, are blocked reversibly by DNDS<sup>8</sup>, have single-channel currents that show pronounced outward rectification with symmetrical chloride concentrations (but not after reconstitution in lipid bilayers<sup>24</sup>), and have an opening probability that is strongly influenced by voltage—they can be opened by large positive potentials in the absence of phosphorylation<sup>14,15</sup>.

During  $\beta$ -adrenoceptor activation in myocytes, a large, maintained outward  $\text{Cl}^-$  current flows almost instantaneously (Fig. 1B) on depolarization (delayed  $\text{K}^+$  current, in contrast, arises over hundreds of milliseconds<sup>1,2,5,6</sup>). This  $\text{Cl}^-$  current must therefore be expected to counter the depolarizing influence of the simultaneously enhanced inward  $\text{Ca}^{2+}$  current<sup>1-4</sup>. The  $\text{Cl}^-$  current should thus speed repolarization of the action potential and so might protect the heart from arrhythmogenic effects of strong sympathetic stimulation. □

**Note added in proof.** Since submission of this manuscript, R. D. Harvey and J. R. Hume<sup>29</sup> have reported a background current, with a reversal potential sensitive to  $[\text{Cl}^-]_i$ , which was elicited in guinea-pig ventricular myocytes by isoprenaline or by forskolin, and was therefore inferred to be regulated by cAMP.

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## Calcitonin gene-related peptide regulates calcium current in heart muscle

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**THE** influx of  $\text{Ca}^{2+}$  due to the transmembrane calcium current,  $I_{\text{Ca}}$ , has a fundamental role in cardiac pacemaker activity, in the action potential plateau and in excitation-contraction coupling. Both sympathetic and parasympathetic neurotransmitters can modulate  $I_{\text{Ca}}$  (ref. 1). Recent studies indicate that in both the cardiovascular<sup>2-7</sup> and the central nervous systems<sup>8,9</sup>, nerve varicosities exist that contain a novel non-adrenergic, non-cholinergic peptide—calcitonin gene-related peptide (CGRP)<sup>10</sup>. Although CGRP is known to exert strong positive inotropic<sup>3-5,7,11</sup> and chronotropic<sup>3,11</sup> effects, as well as to cause vasodilatation<sup>2,6,12,13</sup>, very little is known about the ionic mechanisms of these effects<sup>14,15</sup>. Here we report that CGRP dramatically increases  $I_{\text{Ca}}$  in single heart cells. Although this CGRP-induced increase in  $I_{\text{Ca}}$  resembles the effect of  $\beta$ -adrenergic agonists, our results demonstrate some significant differences between the effects of CGRP and these agonists: (1) the increase due to CGRP cannot be blocked by  $\beta$ -adrenergic antagonists; (2) the CGRP-induced effect is transient; and, (3) CGRP can inhibit isoproterenol-stimulated  $I_{\text{Ca}}$ . Our results provide the first electrophysiological evidence that CGRP can significantly modulate  $I_{\text{Ca}}$  in the heart, and suggest a new additional mechanism for the neurogenic control of cardiac function.

Our initial experiments consistently showed that, in single atrial myocytes from bullfrog hearts, CGRP increased  $I_{\text{Ca}}$  in a potent (threshold CGRP concentration,  $10^{-9}$  M), dose-dependent and reversible fashion. In CGRP of concentration  $3 \times 10^{-7}$  M,  $I_{\text{Ca}}$  recorded at +10 mV increased from  $108 \pm 27$  pA to  $791 \pm 124$  pA (mean  $\pm$  s.e.m.,  $n = 12$ , Fig. 1). Although this ~8-fold increase in  $I_{\text{Ca}}$  resembled the response of these cells to isoprenaline<sup>16</sup>, the CGRP-induced effect could not be blocked by the  $\beta$ -adrenergic antagonist, propranolol ( $3 \times 10^{-7}$  M), indicating that it was not mediated by stimulation of  $\beta$ -adrenoceptors. This is consistent with the recent finding that specific CGRP binding-sites are present in several tissues, including those of the heart<sup>17-19</sup>.

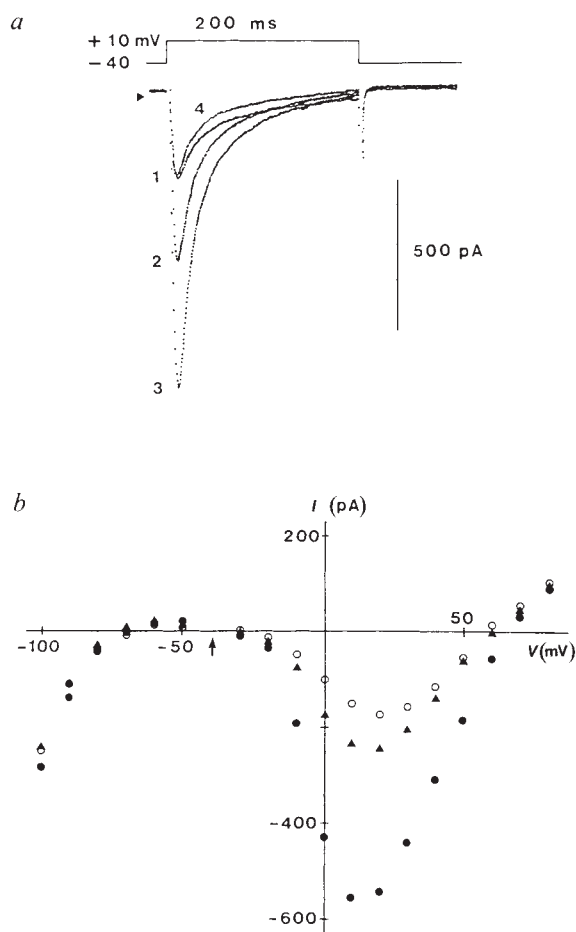
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FIG. 1 Demonstration of the CGRP-induced increase in  $I_{Ca}$  in frog atrial myocytes. *a*, Superimposed tracings of membrane current in control Ringer's solution (1) and CGRP-containing Ringer's solutions (2, 3). CGRP concentrations were  $10^{-8}$  M (2) and  $3 \times 10^{-7}$  M (3).  $I_{Ca}$  records were obtained from a holding potential ( $V_H$ ) of  $-40$  mV by applying 200-ms depolarizing test pulses to  $+10$  mV at 0.2 Hz. Propranolol ( $3 \times 10^{-7}$  M) was present to block  $\beta$ -adrenergic effects. Note that  $I_{Ca}$  increased after the application of CGRP, and that these effects were reversible following washout of the peptide (4). The addition of  $CdCl_2$  ( $3 \times 10^{-4}$  M) blocked these effects completely. The zero current level is indicated by a triangle. *b*,  $I-V$  relation for  $I_{Ca}$  and the inwardly rectifying potassium current ( $I_{K1}$ ).  $V_H$  was  $-40$  mV (shown by an arrow) and 200-ms test pulses were applied in 10-mV increments at 0.2 Hz. The amplitude of  $I_{K1}$ , which was measured at the end of 200-ms test pulse, was not significantly changed by CGRP. Membrane currents were recorded from the same cell in control solutions ( $\circ$ ) and solutions containing CGRP at concentrations of  $10^{-8}$  M ( $\Delta$ ) and  $3 \times 10^{-7}$  M ( $\bullet$ ).

**METHODS.** Atrial myocytes were isolated from bullfrog *Rana catesbeiana*, or from rabbit hearts using methods described previously<sup>20</sup>. In brief, frog atrium was dissected out and cut into pieces (1 mm  $\times$  1 mm) in low  $Ca^{2+}$ -modified frog Ringer's solution (8.0  $\mu$ M  $CaCl_2$ ), and incubated in this solution containing collagenase (240 units  $ml^{-1}$ , Yakult, Japan) and trypsin (3,600 units  $ml^{-1}$ , Type III, Sigma) for 45 min at room temperature. After being rinsed with 0.1% albumin, the tissue was further treated with collagenase (120 units  $ml^{-1}$ ) for 40–60 min. This procedure gave a satisfactory number of viable single cells. Rabbit atrial myocytes were obtained by perfusing a high K-low Cl solution (KB solution) containing collagenase (58–77 units  $ml^{-1}$ ) through the coronary circulation for 20 min at 37 °C (Langendorff perfusion). Composition of KB solution (mM): Glutamic acid mono-K salt (50.0), KCl (25.0), taurine (10.0),  $KH_2PO_4$  (10.0), EGTA (0.5), glucose (10.0), HEPES (10.0),  $MgCl_2$  (3.0), pH 7.4, adjusted with KOH. Electrode solution for frog (mM): K-aspartate (90.0), KCl (20.0), EGTA (5.0), HEPES (5.0), ATP-2Na (3.0),  $MgCl_2$  (2.0), pH 7.2, adjusted with KOH; for rabbit (mM): K-aspartate (120.0), KCl (30.0), EGTA (1.0), HEPES (5.0), ATP-2Na (4.0),  $CaCl_2$  (0.486),  $MgCl_2$  (1.0), pCa 7.0, pH 7.2, adjusted with KOH. In all experiments membrane current was recorded from single isolated myocytes by a single electrode patch-clamp technique in the whole-cell configuration<sup>20,26</sup>. Membrane current was digitized with a sampling speed of 2.5 KHz. Composition of the modified Ringer's solution for frog (mM): NaCl (110.0), KCl (2.5), glucose (10.0), HEPES (5.0),  $CaCl_2$  (2.5),  $MgCl_2$  (5.0), pH 7.4 adjusted with NaOH. Composition of the Tyrode's solution for rabbit (mM): NaCl (135.0), KCl (5.4), glucose (5.5), HEPES (5.0),  $CaCl_2$  (1.8),  $MgCl_2$  (0.5),  $NaH_2PO_4$  (0.33), pH 7.4 adjusted with NaOH. Drugs were dissolved in these solutions and applied by superfusion at  $\sim 1.5$  ml  $min^{-1}$ . The volume of the recording chamber was  $\sim 0.5$  ml. Measurement of the  $I-V$  relation was started 2.5 min after application of each dose of the peptide when  $I_{Ca}$  had reached a steady level. The data



were obtained in 1.5 min. During this period, there was no measurable decline in the current at either high or low doses of CGRP. Drugs used in this study were rat CGRP (Peninsula Laboratories, California), (-)-isoprenaline (Sigma Chemical, Missouri), DL-propranolol (Sigma), cAMP (Sigma), forskolin (Sigma) and cadmium chloride (Sigma).

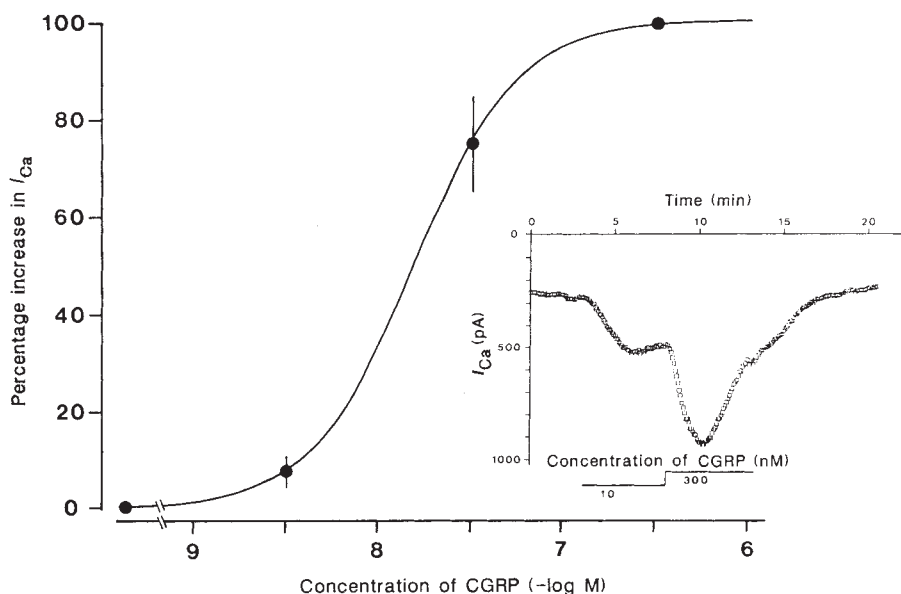


FIG. 2 Dose-response curve for the CGRP-induced enhancement of  $I_{Ca}$  in bullfrog atrial myocytes. In each cell, three different concentrations of CGRP were applied cumulatively by rapidly increasing CGRP concentration just after  $I_{Ca}$  had reached a steady level for each dose. The increase of  $I_{Ca}$ , measured at its maximum level for a particular dose, was expressed as a percentage of the maximum increase ( $\sim 8$ -fold at a CGRP concentration of  $3 \times 10^{-7}$  M) in each cell. The entire experiment was completed within 6 min. Since the effects of CGRP on  $I_{Ca}$  did not decline significantly during this short period (except for the maximal dose), these results provided an indication of the steady-state dose-response relationship. Test pulses of 200 ms to  $+10$  mV from the  $V_H$  of  $-40$  mV were applied at 0.2 Hz. Each point represents the mean  $\pm$  s.e.m. of four different experiments. The solid line shows the best fit Langmuir relationship; the  $ED_{50}$  was  $1.64 \pm 0.55 \times 10^{-8}$  M ( $n=4$ ). Inset, comparison of the time course of the stimulation of  $I_{Ca}$  by low ( $10^{-8}$  M) and high ( $3 \times 10^{-7}$  M) doses of CGRP. When relatively high ( $>10^{-7}$  M) doses were applied for a long period (5 min), the response was found to be transient;  $I_{Ca}$  declines quickly after reaching its maximum.



The current-voltage ( $I$ - $V$ ) relation for  $I_{Ca}$  in Fig. 1*b* illustrates the effects of CGRP at concentrations of  $10^{-8}$  M and  $3 \times 10^{-7}$  M. The increase in  $I_{Ca}$  was dose-dependent without a significant change in its voltage dependence. Moreover, under these conditions, the main effect of CGRP seemed to be on  $I_{Ca}$ , because (1) the inwardly rectifying background current,  $I_{K1}$ , was not changed significantly, as indicated by the lack of effect at potentials negative to  $-50$  mV, (2) the CGRP effect was completely blocked by  $CdCl_2$  ( $3 \times 10^{-4}$  M), and (3) the delayed rectifier  $K^+$ -current,  $I_K$ , is activated very slowly in this preparation<sup>20</sup> and, therefore, is very small when short (200 ms) depolarizations are applied. Under different conditions,  $I_K$ , which is responsible for initiation of repolarization in these cells<sup>20</sup>, can also be increased by CGRP. This effect can be demonstrated in experiments in which much longer depolarizations are used<sup>20</sup> so that  $I_K$  is activated more strongly (data not shown).

We obtained more information about the physiological importance of the CGRP-induced increase in  $I_{Ca}$  by examining its dose-dependence and time course in detail. Figure 2 shows the dose-response relation for the observed effect of CGRP. The threshold CGRP concentration was  $\sim 10^{-9}$  M, and the 50% effective dose ( $ED_{50}$ ) was  $1.64 \pm 0.55 \times 10^{-8}$  M (mean  $\pm$  s.e.m.,  $n = 4$ ). The maximum response ( $\sim 8$ -fold increase) was observed at  $3 \times 10^{-7}$  M ( $n = 4$ ). It is interesting that the dose-dependence of this effect is similar to the dose-dependence of the positive inotropic ( $ED_{50} = 0.6$ – $5.0 \times 10^{-8}$  M)<sup>3,5,11,21</sup> and chronotropic ( $ED_{50} = 0.7$ – $5.0 \times 10^{-8}$  M)<sup>3,11</sup> effects of this peptide. A similar CGRP-induced increase in  $I_{Ca}$  has been recorded in cells from rabbit atrium. In two experiments, CGRP at a concentration of  $3 \times 10^{-7}$  M increased  $I_{Ca}$  at 0 mV by 1.7- and 1.4-fold, respectively (data not shown).

Measurement of the dose-dependent effects of CGRP on  $I_{Ca}$  showed an important difference between this response and the one due to isoprenaline. Although the stimulatory effect of CGRP on  $I_{Ca}$  was well-maintained at CGRP concentrations of  $3 \times 10^{-8}$  M or lower, it was transient at concentrations greater than  $\sim 10^{-7}$  M (Fig. 2, inset; Fig. 3). In contrast, even maximal effects of isoprenaline were well-maintained (Fig. 3). At a CGRP concentration of  $3 \times 10^{-7}$  M,  $I_{Ca}$  reached a maximum after  $\sim 160$  s ( $156 \pm 14$  s, mean  $\pm$  s.e.m.,  $n = 6$ ) and then declined to  $\sim 50\%$  ( $45.6 \pm 5.8\%$ ,  $n = 4$ ) of this value within 3 min (Fig. 3). This decrease was not due to run-down of  $I_{Ca}$  because, after the CGRP effect had declined to a steady level, application of isoprenaline ( $10^{-6}$  M) increased  $I_{Ca}$  substantially (Fig. 3).

Further experiments attempted to obtain information about the intracellular mediator(s) of the CGRP response. CGRP stimulates adenylyl cyclase<sup>5,18,21</sup> in the heart. The enhancement of  $I_{Ca}$  may, therefore, be mediated by increases in intracellular cyclic adenosine-3',5'-monophosphate (cAMP), even though CGRP cannot activate sarcolemmal  $\beta$ -adrenergic receptors. This was tested by either (1) increasing intracellular cAMP by dialysis from the recording pipette, or (2) pre-treating the myocytes with forskolin, a direct activator of adenylyl cyclase. When the cells were perfused intracellularly with cAMP ( $10^{-4}$  M),  $I_{Ca}$  was enhanced maximally<sup>16</sup> ( $1,415 \pm 418$  pA, mean  $\pm$  s.e.m.,  $n = 3$ ). Subsequent application of CGRP ( $3 \times 10^{-7}$  M) did not increase  $I_{Ca}$  any further ( $1,460 \pm 411$  pA,  $n = 3$ , Fig. 4*a*). Forskolin ( $5 \times 10^{-7}$  M) alone did not affect  $I_{Ca}$ ; it significantly potentiated the stimulatory effect of CGRP, however. For example, CGRP ( $10^{-8}$  M) alone gave an increase in  $I_{Ca}$  from 78 pA to 216 pA; in the same cell after pre-treatment with forskolin, CGRP ( $10^{-8}$  M) increased  $I_{Ca}$  to 417 pA (Fig. 4*b*). These results suggest that the enhancement of  $I_{Ca}$  by CGRP is mediated by cAMP, following stimulation of adenylyl cyclase.

One possible explanation for the relatively rapid decline in  $I_{Ca}$  after CGRP-induced enhancement is that the CGRP receptors desensitize quickly<sup>22</sup>. Consistent with this is the finding that, when brief applications of low doses of CGRP ( $\leq 3 \times 10^{-8}$  M) were repeated at intervals of  $>20$  min, the stimulatory effect

was reproducible. Higher doses of CGRP ( $>10^{-7}$  M), however, caused significant desensitization. For example, when  $I_{Ca}$  had declined in the presence of CGRP ( $3 \times 10^{-7}$  M), and the peptide was then washed out and re-applied after 20 or 30 min, the increase in  $I_{Ca}$  was only 30% of that of the first response (data not shown). Moreover, CGRP partially inhibited isoprenaline stimulation of  $I_{Ca}$  (Fig. 4*c*). This inhibitory effect was statistically significant ( $P < 0.001$ ). Further investigation is needed before the intracellular mechanisms for the CGRP-induced transient stimulation of  $I_{Ca}$  can be understood. This stimulation helps to explain previously reported transient effects of CGRP, such as its enhancement of contraction and its stimulation of transmitter release<sup>11,23</sup>.

Our results provide the first direct evidence that CGRP has a significant stimulatory effect on  $I_{Ca}$  in the heart. We show that this effect is reversible, dose-dependent, and resistant to blockade by  $\beta$ -adrenergic antagonists. The CGRP-induced increase in  $I_{Ca}$  seems to be mediated by stimulation of adenylyl cyclase that is coupled to a specific CGRP receptor. The increase in  $I_{Ca}$  can explain the positive inotropic and chronotropic effects of this peptide, because transmembrane calcium influx is essential for both contraction and the pacemaker activity<sup>24</sup>. Combined with previous evidence of CGRP-containing nerve varicosities in the atrial muscle<sup>3,5,7</sup>, and release of this peptide during normal<sup>3,4,7</sup> and ischaemic<sup>25</sup> conditions, the electrophysiological

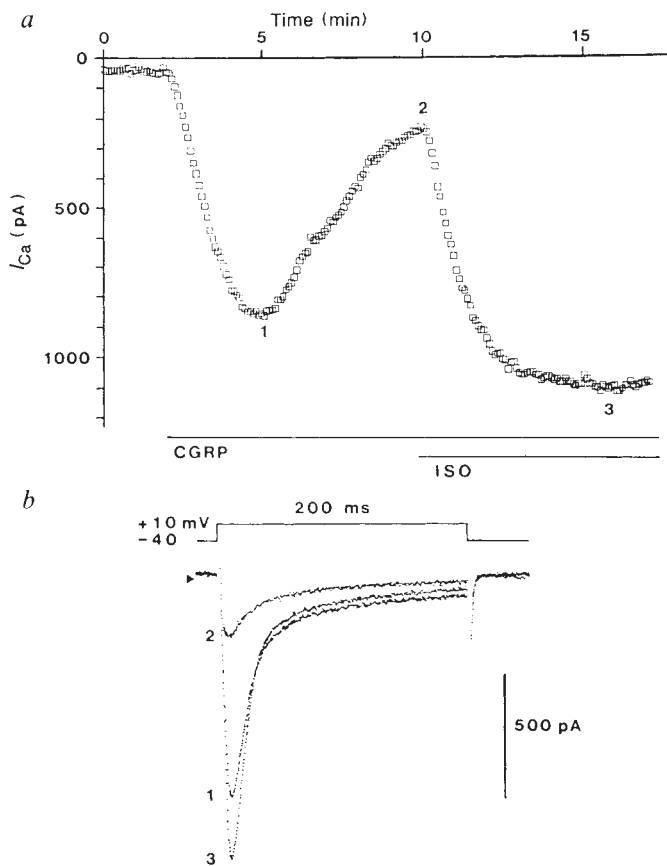


FIG. 3 Comparison of the time-courses of the effects on  $I_{Ca}$  of maximal doses of CGRP ( $3 \times 10^{-7}$  M) and isoprenaline (ISO) ( $10^{-6}$  M). *a*, Time courses of the change in the peak size of  $I_{Ca}$ . The horizontal bars show the period during which each drug was applied. Note that the CGRP effect is transient whereas that of isoprenaline is maintained. *b*, Superimposed records, taken at the time indicated in *a*. The zero current level is indicated by a triangle. CGRP increased  $I_{Ca}$  (from  $125 \pm 35$  pA to  $754 \pm 121$  pA, mean  $\pm$  s.e.m.,  $n = 8$ ) (1), but this peak response rapidly decayed (2). After the CGRP-induced decline of  $I_{Ca}$  had reached a steady level, isoprenaline was added in the presence of CGRP. As shown in *a*, isoprenaline still gave a significant increase in  $I_{Ca}$ ;  $I_{Ca}$  exceeded that recorded previously in the presence of CGRP (3). The same result was obtained when isoprenaline was applied in the absence of CGRP, after the decline of  $I_{Ca}$ .

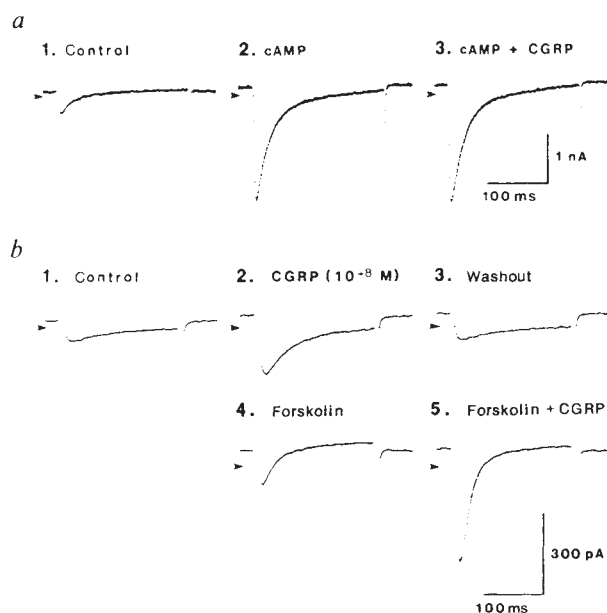
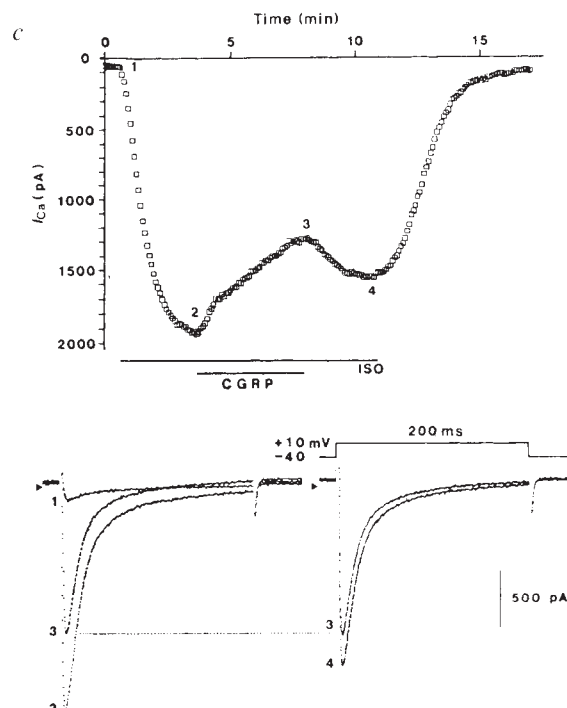


FIG. 4 *a*, Tests for involvement of intracellular cAMP in the CGRP-induced increase in  $I_{Ca}$ . Cyclic AMP ( $10^{-4}$  M) was included in the pipette before the whole-cell impalement was made, and the control record (1) was taken immediately after the impalement. Cyclic AMP maximally increased  $I_{Ca}$  (2) and the subsequent application of CGRP ( $3 \times 10^{-7}$  M) did not increase  $I_{Ca}$  any further (3). *b*, Potentiation of the effect of CGRP by forskolin ( $5 \times 10^{-7}$  M). A low dose of CGRP ( $10^{-8}$  M) was applied repeatedly at intervals of 30 min. Forskolin was applied 5 min before the second application of CGRP. Forskolin alone did not increase  $I_{Ca}$  (4), but it strongly potentiated the effect of the subsequent application of CGRP (2, 5).  $I_{Ca}$  records in the presence of CGRP were taken after it had increased maximally at each peptide application. Triangles denote the zero current level in all panels. *c*, Inhibitory effect of



CGRP ( $3 \times 10^{-7}$  M) on  $I_{Ca}$  stimulated by isoprenaline (ISO) ( $10^{-6}$  M). Upper panel shows the time course of the changes in  $I_{Ca}$ . Lower panel shows raw data recorded at the times marked by the numbers in the upper panel. Isoprenaline ( $10^{-6}$  M) increased  $I_{Ca}$  maximally within 2 min (2). This effect was persistent; there was no detectable decay 3 min after the maximal level was reached ( $98.0 \pm 3.2\%$ , mean  $\pm$  s.e.m.,  $n=4$ , Fig. 3a). In contrast,  $I_{Ca}$  declined quickly when CGRP ( $3 \times 10^{-7}$  M) was added just after it had reached its maximum size in the presence of isoprenaline ( $10^{-6}$  M) (3); 3 min after the application of CGRP,  $I_{Ca}$  had decreased to  $72.1 \pm 3.4\%$  of the initial maximum size ( $n=5$ ,  $P<0.001$ , compared with the value of  $I_{Ca}$  in the absence of CGRP). This inhibitory effect was partly reversed when CGRP was washed out (4).

actions of CGRP that we have identified provide important new insights into neurogenic control of the heart in both normal<sup>3,4,7</sup> and pathological<sup>25</sup> conditions. □

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## Altering DNA-binding specificity of GAL4 requires sequences adjacent to the zinc finger

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MANY eukaryotic proteins involved in transcriptional regulation contain within their DNA-binding domains a polypeptide loop (the zinc finger) which interacts with DNA<sup>1,2</sup>. In proteins possessing multiple zinc fingers, including TFIIIA<sup>3</sup>, Sp1<sup>4,5</sup>, SWI5<sup>6</sup> and oestrogen/glucocorticoid receptors<sup>7</sup>, the region containing the zinc fingers confers DNA-binding specificity. By contrast, our results demonstrate that all but one of the 28 amino acids encompassing the single zinc-finger region of GAL4, the yeast transcriptional activator, can be replaced with the analogous zinc-finger region from another yeast-activator protein, PPR1, without changing the DNA-binding specificity of GAL4. A 14-amino-acid region adjacent to the zinc finger is necessary for determining specific recognition of DNA sequences.

To determine the role of the GAL4 zinc finger in specific DNA binding, increasing segments of the 28-amino-acid cysteine-rich zinc-finger region of GAL4 protein have been

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FIG. 1 *MEL1* activation and DNA-binding activities of the GAL4-PPR1 hybrid proteins. PPR1 sequences are boxed. The six conserved cysteines are double underlined. Two pairs of cysteines (Cys<sub>1</sub> and Cys<sub>2</sub>, Cys<sub>3</sub> and Cys<sub>4</sub>) are thought to coordinate an atom of zinc to form the zinc finger. Numbers on the side refer to the position in the protein of the first amino acid in the line. Numbers on top refer to the position in GAL4 of the last PPR1 amino acid in the hybrid protein. Lys23 in GAP3Lys23 is underlined. The consensus sequence includes amino acids which are conserved in at least half of the 10 proteins from the C<sub>6</sub> class including GAL4<sup>13</sup>, LAC9<sup>23</sup>, qa-1F, QUTA<sup>24</sup>, PPR1<sup>9</sup>, ARGRII, HAP1, LEU3, MAL6 and PDR1<sup>25</sup>. Standard one letter codes are used, plus Z for the hydrophobic amino acids W, I, V and L, and B for basic amino acids R or K. *MEL1* ( $\alpha$ -galactosidase) activities of the hybrids are shown as a percentage of the activation by wild-type GAL4. The DNA-binding behaviour is shown as either + or - determined by electrophoretic mobility shift assays (Fig. 2).

METHODS. The starting plasmid, GAL4/pUC119, consisted of a *Bam*HI/*Hind*III fragment containing the wild-type GAL4 gene cloned into pUC119<sup>26</sup>. Single-stranded DNAs were prepared as described<sup>26</sup> and used as templates for site-directed mutagenesis using an Amersham *in vitro* mutagenesis kit. Primers used to direct mutagenesis were synthesized on an Applied Biosystems Model 380B DNA synthesizer. The sequences of the primers are available upon request. The *Bam*HI-*Xho*I fragments from the hybrids were cloned into the 2  $\mu$  plasmid GAL4/YEp351 containing the selectable marker *LEU2* gene, replacing the wild-type *Bam*HI-*Xho*I fragment. The nine amino acids N-terminal to the first cysteine of the zinc finger of GAL4 were converted to the analogous PPR1 sequence. The GAL4 DNA sequences of the GAP4 hybrid encoding into the first nine amino acids on a *Bam*HI-*Sph*I fragment were replaced with the PPR1 sequences on a *Bam*HI-*Sph*I fragment. All sequence changes by mutagenesis were confirmed by sequencing<sup>27</sup>. The PPR1 gene contained on a *Pvu*II-*Pvu*II fragment from the plasmid

|                       |    |           |                 |                     |        |        |                |        |     |   | Activation<br>of<br>MEL1 | Binding to<br>UAS <sub>G</sub> UAS <sub>A</sub> |  |
|-----------------------|----|-----------|-----------------|---------------------|--------|--------|----------------|--------|-----|---|--------------------------|-------------------------------------------------|--|
|                       |    |           | 10              | 26                  | 32     | 38     |                | 52     |     |   |                          |                                                 |  |
| GAL4                  | 1  | M         | KLLSSIEQA       | CDICRLKKLKCSKEPK    | KCAKCL | KNNWEC | RYSPTKRSPLTRA  | HLTEVE | 100 | + | -                        | -                                               |  |
| GAP1                  | 1  | M         | KLLSSIEQA       | CKRCRLKKIKCDQEFF    | KCAKCL | KNNWEC | RYSPTKRSPLTRA  | HLTEVE | 0   | - | -                        | -                                               |  |
| GAP2                  | 1  | M         | KLLSSIEQA       | CKRCRLKKIKCDQEFF    | SCKRCA | KNNWEC | RYSPTKRSPLTRA  | HLTEVE | 0   | - | -                        | -                                               |  |
| GAP3                  | 1  | M         | KLLSSIEQA       | CKRCRLKKIKCDQEFF    | SCKRCA | KLEVPC | RYSPTKRSPLTRA  | HLTEVE | 1   | - | -                        | -                                               |  |
| GAP3 <sup>Lys23</sup> | 1  | M         | KLLSSIEQA       | CKRCRLKKIKCDQEFF    | SCKRCA | KLEVPC | RYSPTKRSPLTRA  | HLTEVE | 74  | + | -                        | -                                               |  |
| GAL4N                 | 1  | M         | IGISKSRTA       | CDICRLKKLKCSKEPK    | KCAKCL | KNNWEC | RYSPTKRSPLTRA  | HLTEVE | 124 | + | -                        | -                                               |  |
| GAP4N                 | 1  | M         | IGISKSRTA       | CKRCRLKKIKCDQEFF    | SCKRCA | KLEVPC | VSLDPATGKDVPRS | HLTEVE | 0   | - | +                        | +                                               |  |
| PPR1                  | 24 | N         | IGISKSRTA       | CKRCRLKKIKCDQEFF    | SCKRCA | KLEVPC | VSLDPATGKDVPRS | YVFFLE | 0   | - | +                        | +                                               |  |
| CONSENSUS             | -  | --R-BZ--A | CD-CR-BKZKCD--P | -C--C-              | K-NZ-C | -Y---- | BB-----        | ----LE |     |   |                          |                                                 |  |
|                       |    |           |                 | ← ZINC FINGER →     |        |        |                |        |     |   |                          |                                                 |  |
|                       |    |           |                 | ← CYS-RICH REGION → |        |        |                |        |     |   |                          |                                                 |  |

PPR1/pUC8 (provided by R. Losson) was cloned into the *Sma*I site of YEp351. A *Bam*HI-*Hind*III fragment containing GAP3 was cloned into vector YCp50f1. This plasmid contains the *f1* ori region from plasmid pVT100-L<sup>28</sup> in YCp50. The yeast strain SC30 (*a gal4<sup>Δ</sup> ura3-52 leu2-3 112*) containing GAP3/YCp50f1 was plated on to minimal media lacking uracil containing 2% galactose. Plasmids from revertants were isolated from yeast<sup>29</sup>, transformed into *E. coli*, checked by restriction enzyme digests, and sequenced<sup>27,30</sup>. Putative revertant plasmids were transformed into YJ0 (*a gal4<sup>Δ</sup> ura3-52 leu2-3 112 his3<sup>Δ</sup> ade1 gal80<sup>Δ</sup> MEL1*). The *Bam*HI-*Xho*I fragment of the revertant was cloned into plasmid GAL4/351. The  $\alpha$ -galactosidase (*MEL1*) assay was essentially as reported<sup>10</sup>. The YJ0 strain carrying the GAL4, GAP or PPR1 plasmids was grown in minimal media lacking leucine. The carbon source was 3% glycerol and 2% lactic acid. All plasmids were tested from level of production of GAP proteins in a B80<sup>8</sup> strain (*a GAL4 GAL80<sup>8</sup>-100 ura3-52 leu2-3 112 MEL1*) by assaying growth rate on galactose<sup>17</sup>. PPR1 or YEp351 plasmids did not allow the strain to grow on galactose.

replaced, by *in vitro* mutagenesis, with the analogous sequences of PPR1, an activator of two genes involved in pyrimidine biosynthesis, *URA1* and *URA3* (refs 8, 9). The plasmids encoding the hybrid proteins, designated GAP or GAL4N (Fig. 1) were tested in a *gal4<sup>Δ</sup>* strain for their ability to activate expression of the *MEL1* ( $\alpha$ -galactosidase) gene which is normally under the control of GAL4. Neither GAP1 nor GAP2 activated *MEL1* expression. The yeast strain containing the plasmid encoding GAP3, in which all of the cysteine-rich region of GAL4 was replaced, possessed 1% of the *MEL1* activity of the strain containing the GAL4-encoded plasmid. A *gal4<sup>Δ</sup>* strain has less than 0.1% background activity<sup>10</sup>.

Hybrid protein levels were determined by assaying another function of GAL4, namely its ability to interact through the C terminus<sup>11,12</sup> with the GAL80<sup>8</sup> negative regulatory protein (Fig. 1 legend). All of the hybrid proteins used in this study were stably produced at high enough levels to titrate out the repressive effects of GAL80<sup>8</sup> protein, reflecting at least 50% wild-type protein levels. The low or negligible activity of GAP1, GAP2 and GAP3 is, therefore, most probably due to a disrupted DNA-binding structure and not low protein stability or production.

A mutation in the GAP3 gene was found which conditioned higher levels of GAL and *MEL1* gene expression. Mutants of a *gal4<sup>Δ</sup>* strain containing GAP3 were selected on plates containing galactose as the sole carbon source. A GAP3 mutant which grew well on galactose was found to be a C to A transversion at nucleotide 509 (by the numbering of Laughon and Gesteland<sup>13</sup>) resulting in a predicted change of Gln 23 to Lys near the tip of the zinc finger. Lysine is the amino acid found at this position in GAL4. On a multicopy plasmid, GAP3Lys23 acti-

vates 74% of the level of *MEL1* gene expression that is activated by GAL4.

The DNA binding properties of the hybrid proteins were studied directly by electrophoretic mobility shift assays<sup>14,15</sup> using either a GAL4 DNA-binding site, UAS<sub>G</sub>, or a PPR1 DNA-binding site, UAS<sub>U</sub>. This *in vitro* assessment of the hybrid proteins was important, because transcriptional activation and DNA binding are separable functions<sup>16</sup>. Extracts containing GAP1, GAP2 or GAP3 proteins did not form detectable complexes with either UAS<sub>G</sub> or UAS<sub>U</sub> (data not shown). However, specific binding by GAP3Lys23 to UAS<sub>G</sub> was detected (Fig. 2a, lanes 4 and 5). The putative GAP3Lys23-UAS<sub>G</sub> complex reacted with antiserum against GAL4 protein<sup>17</sup> (Fig. 2a, lane 6), but not with preimmune serum (Fig. 2a, lane 5), confirming that the complex contained the GAL4 hybrid protein. Thus, the GAL4 protein containing most of the PPR1 zinc-finger region retained GAL4 DNA-binding specificity and ability to activate *MEL1* to high levels.

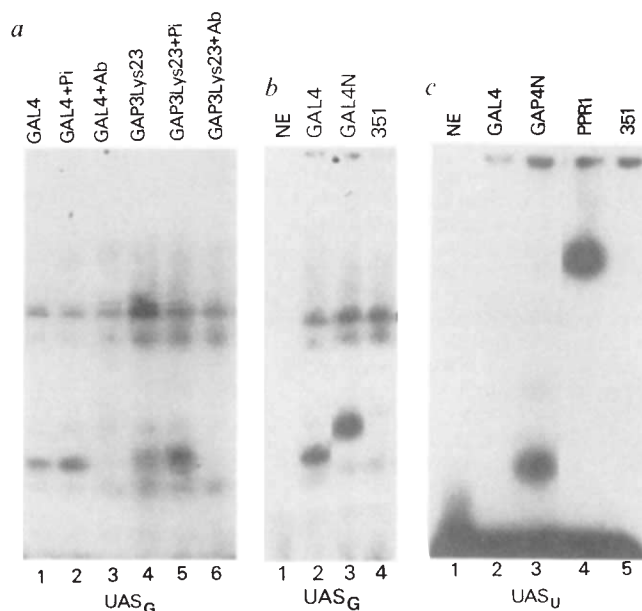
The contribution to DNA binding specificity by amino acids directly preceding the zinc finger was determined by replacing the 9 N-terminal amino acids of GAL4 with the analogous PPR1 sequences. The hybrid GAL4N activated the *MEL1* gene to a level 124% that of wild-type GAL4 and retained the DNA binding specificity of GAL4 (Fig. 2b, lane 3) demonstrating that these 9 amino acids are not involved in DNA-binding specificity.

To locate the sequences required for specific recognition of DNA, we converted an additional 14-amino-acid region immediately following the sixth cysteine of the zinc-finger region to give the hybrid GAP4N. These 14 amino acids of PPR1 switched the DNA-binding specificity of GAL4 to that of PPR1 as GAP4N bound specifically to UAS<sub>U</sub> (Fig. 2c, lane 3) but did not bind



FIG. 2 DNA-binding specificity of GAL4-PPR1 hybrid proteins. *a*, Binding of GAP3Lys23 to UAS<sub>G</sub> and specific titration of the GAP3Lys23-UAS<sub>G</sub> complex with anti-GAL4 antiserum. UAS<sub>G</sub> was preincubated for 30 min with extracts containing GAL4 (lanes 1–3), or GAP3Lys23 (lanes 4–6). Then 1 µl of preimmune serum (Pi, lanes 2 and 5) or 1 µl of immune serum (Ab, lanes 3 and 6) was added to the tubes. After an additional 30 min incubation, the protein-DNA complexes were resolved on an agarose gel. The arrowhead points to the GAL4- and GAP3Lys23-specific complexes. *b*, Binding of GAL4N to UAS<sub>G</sub> was incubated with buffer alone (lane 1, NE) or with extracts containing GAL4 (lane 2), GAL4N (lane 3) or YEp351 (lane 4). The arrowhead points to the GAL4N-UAS<sub>G</sub> complex. *c*, Binding of GAP4N to UAS<sub>U</sub>. UAS<sub>U</sub> was incubated with buffer alone (lane 1, NE) or with extracts containing GAL4 (lane 2), GAP4N (lane 3), PPR1 (lane 4) or YEp351 (lane 5). The arrowhead points to the GAP4N-UAS<sub>U</sub> complex.

**METHODS.** Yeast extracts were prepared using a modification of the procedure of Bram and Kornberg<sup>31</sup>. All steps were performed at 0–4 °C and buffer A(50) did not include pepstatin A or leupeptin. Typically, a 40 ml culture of yeast cells was grown at 30 °C to 3–5 × 10<sup>7</sup> cells ml<sup>-1</sup>. The cells were collected by centrifugation (10 min, 3,000g) and resuspended in buffer A(50) plus 0.48 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. The cells were lysed by adding acid-washed glass beads (0.45 mm) and vortexing for 4 × 30 s at maximum speed. The resulting crude extract was centrifuged at 5,000g for 10 min and the supernatant collected. Solid ammonium sulphate (0.46 g ml<sup>-1</sup>) was immediately added to the supernatant. The solution was gently mixed until the ammonium sulphate was completely dissolved. The precipitated protein was collected by centrifugation at 10,000g for 15 min and resuspended in 200 µl buffer A(50). The resuspended protein was dialysed against 500 volumes of buffer A(50) for at least 2 h. The resulting extracts were stored at –20 °C and contained 8–10 mg protein per ml. Synthetic oligonucleotides containing the sequence 5'-AATTCGGGTGACAGCCCTCCGAAGGGTAC-3' and 5'-CCTTCGGAGGGTGTACACCG-3' (UAS<sub>G</sub>) or 5'-AATTCATTCGGTAATCTCCGAACGGTAC-3' and 5'-CGTTCGGAGATTACCGAATG-3' (UAS<sub>U</sub>) were annealed and labelled with [γ-<sup>32</sup>P]ATP and T4 polynucleotide kinase. Each binding reaction contained buffer A(50) without protease inhibitors, 0.75 µg of sonicated salmon sperm DNA, 0.8 ng [<sup>32</sup>P]DNA, and 5–15 µl yeast extract in a total volume of 20 µl. The reactions were incubated on ice for 45 min



followed by addition of 1 µl sample dye. The reactions were immediately loaded on to a 2.5% NuSieve agarose (FMC Bioproducts) + 0.5% low melting temperature agarose (International Biotechnologies) gel in 0.5 × TBE (0.045 M Tris-borate, pH 8.0, 0.045 M boric acid, 0.001 M EDTA) and electrophoresed at 200 V at 3 °C. After electrophoresis the gel was fixed for 30 min in 10% trichloroacetic acid, blotted dry between paper towels overnight, and dried on a vacuum gel dryer with 45 °C heat. Autoradiography was performed with Kodak X-Omat film at –70 °C with an intensifying screen for 1–3 days.

the UAS<sub>G</sub> (data not shown). The putative GAP4N-UAS<sub>U</sub> complex reacted with GAL4 immune but not preimmune sera (data not shown) confirming that GAL4 protein sequence was in the complex. As expected, GAP4N failed to activate *MEL1* expression, indicating that it did not bind to and activate at UAS<sub>G</sub>, to even low levels. The binding activity of GAP4N demonstrates that sequences necessary for the specific recognition of UAS<sub>U</sub> by PPR1 are encoded in the 14 amino acids flanking the sixth cysteine of the zinc-finger region.

The zinc-finger structure has been shown to confer the ability of some transcriptional activator proteins to interact specifically with DNA. We show that for two members of the C<sub>6</sub> class of zinc-finger proteins<sup>2</sup>, most of the important determinants of specificity are not located within the zinc finger. The zinc fingers in at least some of these proteins may perform a common function such as non-sequence-specific interactions with the DNA. In this regard, a region of the adenovirus E1a protein<sup>18</sup> containing one zinc finger and the second zinc finger in the glucocorticoid/oestrogen receptors<sup>7</sup> have been implicated in non-sequence-specific interactions with DNA. Both the second finger from the steroid receptors and the C<sub>6</sub> class fingers contain several basic residues found primarily on one half of the finger, which may make contacts with the DNA phosphate-backbone.

We have shown that amino-acid sequences necessary for specific recognition of UAS<sub>U</sub> are located within the 14 amino acids following the sixth cysteine. Mutations within this region of GAL4 abolish DNA binding<sup>19,20</sup>. Two differences distinguish these 14 amino acids from the three amino acids important in determining the specificity of the glucocorticoid/oestrogen receptors<sup>21</sup>. The three amino acids are located close to the second pair of cysteines of the first zinc finger, whereas the 14 amino acids of GAL4 are located a greater distance (seven amino acids) after the second pair of cysteines. Furthermore, most of the 14 amino-acid region of GAL4 has been predicted to form a random

structure<sup>22</sup>, whereas the three amino acids important in receptor binding are located within a proposed α-helix.

Homology within the 14 amino acids between two evolutionarily related proteins which recognize the same DNA sequence, *GAL4* and *LAC9* (the *GAL4* homologue from *Kluyveromyces fragilis*) indicated the importance of these sequences in DNA binding specificity<sup>23</sup>. By contrast, there is little homology between *GAL4* and other proteins in the C<sub>6</sub> class within this region, even though all C<sub>6</sub> class proteins have homology within the cysteine-rich region (Fig. 1, consensus). Interestingly, two other evolutionarily-related proteins qa-1F and QUTA isolated from two different fungi share a region of extensive homology in approximately the same position as the specificity region of *GAL4* and *LAC9* (ref. 24).

Our results suggest we can roughly partition the *GAL4* DNA-binding region into two functional domains, supporting an earlier model of how C<sub>6</sub> proteins bind DNA<sup>23</sup>. It should be emphasized, however, that the DNA-binding region may have complex interactions between different parts of itself which preclude complete separation of a functional domain. In support of this, two mutations in *gal4<sup>A</sup>*—found 10 and 26 amino acids C-terminal to the zinc finger—showed growth correction on galactose in the presence of high concentrations of Zn(II). It was hypothesized that these alterations were manifestations of direct interaction between amino acids in the zinc finger and those downstream<sup>20</sup>. It is possible that Lys23 in the GAP3 mutant makes a structural contact with another part of the *GAL4* protein or contributes to DNA-binding specificity. Further replacements of the DNA-binding domain of *GAL4* are underway to locate the key amino acids determining binding specificity. □

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## Two tobacco DNA-binding proteins with homology to the nuclear factor CREB

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THE 35S promoter of the cauliflower mosaic virus (CaMV) contains a tandem repeat of the sequence TGACG in the region -83 to -63. This 21-base pair (bp) sequence, called *as-1*, is involved in root expression of the 35S promoter. When inserted in a promoter of a gene expressed specifically in photosynthetic tissues, *as-1* confers high level expression in roots<sup>1,2</sup>. We have described a factor, ASF-1, that binds specifically to *as-1* *in vitro*. There is a good correlation between ASF-1 binding affinity to *as-1* related sequences *in vitro* and the function of these sequences *in vivo*. These results strongly suggest that ASF-1 is responsible for the function of *as-1* (ref. 13). Here we report the isolation of tobacco complementary DNA clones encoding two TGACG-sequence-specific binding-proteins (TGA1a and TGA1b). Sequence analysis of the cDNA clones shows that both proteins contain a basic region that shows high homology to a stretch of basic amino acids in the nuclear factors CREB, GCN4, and c-Jun<sup>1-4</sup> to a 'leucine-zipper' region<sup>5</sup>. On the basis of binding specificity we propose TGA1a to be a good candidate for ASF-1.

We have reported previously that tobacco nuclear extract contains a factor, ASF-1, that binds to the *as-1* sequence of the 35S promoter<sup>13</sup>. Mutations in the TGACG motifs of *as-1* leads to a drastic decrease in this binding activity. The results shown in Fig. 1a confirmed these findings and show that at the extract concentration used, two DNA-protein complexes were obtained with *as-1*. Only the faster-migrating complex (marked by an arrow head), however, is seen at a lower extract concentration<sup>13</sup>. This faster-migrating complex was also formed with two other TGACG-containing sequences, *hex1* and *nos1*, present in the upstream region of the wheat histone H3 gene and the promoter of the gene encoding nopaline synthase, respectively<sup>6,7</sup> (Fig. 1a). In both cases, mutations in the TGACG motifs reduced ASF-1 binding. An additional DNA-protein complex (marked

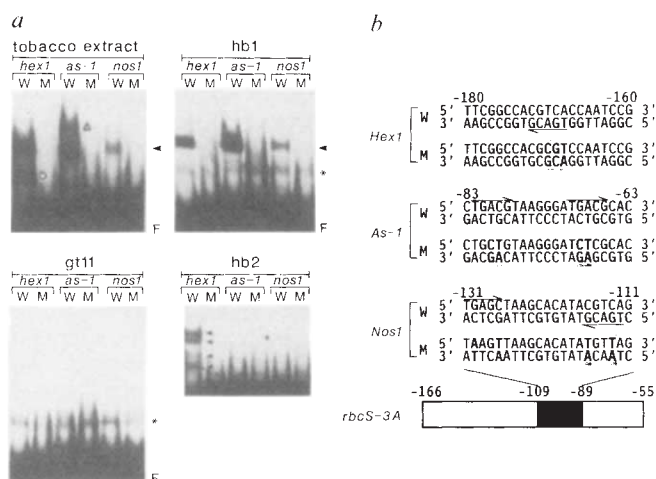


FIG. 1. DNA binding specificity of ASF-1, TGA1a, and TGA1b. *a*, Gel retardation assays<sup>18</sup> with tobacco nuclear extract and lysogen extracts from hb1, hb2, and λgt11. For hb2, the lower part of gel was cut off before autoradiography because the signals of the specific complexes were very weak compared with the signals from the free DNA. The results are presented so that the mobilities of the complexes are directly comparable with reference to the position of free DNA. Arrow head, specific DNA-protein complex; asterisk, non-specific complex in lysogen extract; F, free DNA; ○, *hex1*-specific complex; △, presumptive complex with two molecules of ASF-1 (ref. 13); W, wild-type sequence; M, mutant sequence. *b*, Binding site probes used in *a*. The -109 to -89 region of the *rbcS-3A* upstream fragment (-166 to -55) was substituted with the wild-type or mutant sequences (ref. 13 and E.L., unpublished data). The mutated bases are shaded. Lines with arrows indicate TGACG motifs or a similar motif, TGACG. The numbers over the wild-type sequences show the nucleotide positions in the original promoters (wheat histone H3 promoter, CaMV 35S promoter, and T-DNA nopaline synthase promoter for *hex1*, *as-1*, and *nos1*, respectively (refs 6, 7 and 13).

METHODS. Poly(A)<sup>+</sup> RNA was prepared from leaves of tobacco (*Nicotiana tabacum* cv. SR1) adapted in the dark for two days<sup>6</sup>. A random-primed cDNA library was constructed in λgt11 vectors. The primary library was plated at a density of ~15,000 plaque-forming units per 15-cm plate. After 6-h incubation at 37 °C, IPTG-impregnated nitrocellulose filters were layered on top of the agar and the plates were further incubated at 37 °C for 6 h. The filters were processed at 4 °C by lifting and incubating them in B buffer (20 mM HEPES-KOH pH 7.5, 40 mM KCl, 1 mM EDTA, 10% glycerol, 0.5 mM DTT) supplemented with 5% non-fat dry milk for 2 h. After a brief washing in B buffer the filters were incubated for 4 h in B buffer containing the labelled binding probe (2.5 ng ml<sup>-1</sup>, 7 × 10<sup>5</sup> c.p.m. ml<sup>-1</sup>) and 5 μg ml<sup>-1</sup> sonicated and denatured salmon testis DNA. Synthetic double-stranded *hex1* oligonucleotides (21-bp sequence as shown in *b*) were concatemerized with T4 DNA ligase until the concatemers contained, on average eight copies of the sequence. The concatemers were labelled by nick translation and used as the binding probe. After incubation with the probe the filters were washed in B buffer for 50 min, briefly dried, and then autoradiographed. For gel retardation assays, lysogen extract and tobacco nuclear extract were prepared as described (refs 8 and 13). The probes were labelled at the *Hind*III sites (-166) by fill-in reaction with the Klenow fragment enzyme. After digestion of the labelled DNAs with *Bst*XI (-55), the probes were purified by polyacrylamide gel electrophoresis. The assay mixture contained lysogen extract (6.8 μg of protein) or tobacco extract (7.5 μg of protein), 0.1 ng of binding probe (4 × 10<sup>4</sup> c.p.m.), and 3 μg of poly(dI-dC) in 10 μl of B buffer supplemented with 0.8 mM phenylmethylsulphonyl fluoride. The mixture was incubated for 20 min at room temperature, before being loaded on to a non-denaturing 0.4% agarose-3% polyacrylamide composite gel. After electrophoresis, the gel was dried and autoradiographed with an intensifying screen. The exposure time was 12 h, except for the gel for hb2 that was exposed for 9 days after the free DNA portion was cut off.

by an open circle) obtained with the *hex1* probe is competed by only *hex1*, but not by *as-1* or *nos1* (not shown).

To isolate cDNA clones encoding the DNA-binding proteins specific for the sequence TGACG, we screened a tobacco cDNA expression library using concatemers of *hex1* as a probe, as previously described<sup>8,14</sup>. Five positive clones were obtained from a primary library consisting of 6 × 10<sup>4</sup> recombinants. All five clones showed reproducible binding to *hex1*. The five λ clones, named hb1, -2, -3, -5, and -6, were divided into two groups according to the nucleotide sequences of their cDNA inserts. The protein encoded by the first group, which included hb1, -3, and -5, was named TGA1a and the protein encoded by the second group, which included hb2 and -6, was named TGA1b.

The binding specificities of TGA1a and TGA1b were confirmed by gel retardation assays with the lysogen extract of



hb1 and hb2, respectively (Fig. 1a). The lysogen extract of hb1 gives two DNA-protein complexes, one of them is specific to each of the wild-type probes, *hex1*, *as-1*, and *nos1*, but not to the mutant probes (Fig. 1a, marked by an arrow head). The other complex (marked by an asterisk in Fig. 1a) is also seen with the lysogen extract of the vector  $\lambda$ gt11 and is, therefore, non-specific. These results demonstrate that TGA1a had the same specificity as ASF-1 for binding to these sequences. We consider TGA1a, therefore, to be a good candidate for ASF-1. The lysogen extract of hb2 gave four complexes specific only to the *hex1* wild-type probe (Fig. 1a, marked by arrow heads). These bands may represent different states of the complexes or proteolytic products. Because TGA1b bound only to *hex1*, TGA1b could correspond to the *hex1*-specific complex (marked by an open circle in Fig. 1a). The difference in binding specificity between TGA1a and TGA1b could be due to differences in the nucleotide sequence flanking the TGACG motif.

Sequence analysis of the inserts of hb1, -3, and -5, which encode TGA1a, shows that these clones seem to have been derived from the same messenger RNA species (Fig. 2a). The longest open reading frame (ORF) starting with a methionine is 1,077 bp, corresponding to 359 amino-acid residues with a relative molecular mass ( $M_r$ ) of 40,694. Because no other significantly long ORF is found, we conclude that this ORF encodes TGA1a. The ORF in hb1 is out-of-frame from the reading frame of the *lacZ* gene. Surprisingly, in gel retardation assays the specific DNA-protein complex obtained with the lysogen extract of hb1 migrated similarly as that obtained with ASF-1 from tobacco extract (Fig. 1a). We presume that the cDNA insert had a cryptic sequence that functioned as a translation initiation site in *E. coli*. These results, as well as the results of northern blot analysis, indicate that the coding region of the cDNA insert in hb1 was near full-length. The deduced polypeptide has an acidic region (amino-acid residues 5-52; 12 acidic and 4 basic),

**a**

|      |                                                                                                                 |                                                         |      |
|------|-----------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|------|
| 1    | GAATCTTCAACGTCACCAATTTGCTGCTCAAGAAGG                                                                            | ATG GGT ATA TGC GAT CCG ATC CAT CAA CTT GGC ATG TGG GAT | 82   |
| 15   | M G I C D P I H Q L G M W D                                                                                     |                                                         | 14   |
| 83   | GAT TTC AAT AGT AGT TTC CCA AGT ACA TCG GCA ACC ATG ATT TTA GAA GAT GAT AAA TGC CTA GAG GAC CAG ATA CCA ATT ATG |                                                         | 166  |
| 15   | D F N S S F P S T S A T M I L E V D K C L E D Q I P I M                                                         |                                                         | 42   |
| 167  | GAG AAA AGA CTA GAC AAC GAG CAC ACT TCG CAT GGA ACA GGA GGT ACT TCT AAC GAA TAT GAA CCG GAA ACA AGT AAA         |                                                         | 250  |
| 43   | E K R L D N E T E D T S H G T V G T S N R Y E P E T S K                                                         |                                                         | 70   |
| 251  | CCC GTC GAG AAG GTA CTT AGA CGT CTT GCA CAA AAC CGC GAG GCT GCT GCT AAA AGC CGT TTG CGG AAG AAG GCC TAT GTT CAG |                                                         | 334  |
| 71   | P V E K R V L R E K Q N R E K S R L R K K A Y V V Q                                                             |                                                         | 98   |
| 335  | CAG TTA GAA AAT AGT AAA TTG AAG CTG ATT CAA CTG GAA CAA GAA CTA GAA CGC GCC AGA AAA CAG GGC ATG TGT GTA GGT GGT |                                                         | 418  |
| 99   | Q L E N S K L K L I Q L E Q E L E R A R K Q G M C V G G                                                         |                                                         | 126  |
| 419  | GGT GTA GAT GCT AGC CAG CTA AGT TAC TCT GGA ACC GCT AGC TCA GGA ACT GCT GTA TTT GAT ATG GAG TAT GGT CAC TGG GTA |                                                         | 502  |
| 127  | G V D A S Q L S Y S G T A S S G T A V F D M E Y G H W V                                                         |                                                         | 154  |
| 503  | GAA GAG CAA ACT AGA CAA ACA AAT GAC TTA AGG ATT GCT TTG CAT TCT CAA ATT GGT GAA CGC GAA TTG CGC ATT ATT GTT GAT |                                                         | 586  |
| 155  | E E Q T H D L R I A L H S E A E L R I I V D                                                                     |                                                         | 182  |
| 587  | GGT TAC CTG AAC CAC TAC TTT GAT CTC TCT CGC ATG AAA GCT ACG GCT AAA GCT GAT GTC TAC TAT TCT GGT ATG             |                                                         | 670  |
| 183  | G Y L N H Y F D L F R M K A T A C A G D V L Y I M S G M                                                         |                                                         | 210  |
| 671  | TGG AAG ACA TCT GCC GAG CGC TTT TTC ATG TGG ATT GGA GGG TTT CGG CCA TCC GAG CTT CTA AAG GTT CTC ACA CCG CAT CTT |                                                         | 754  |
| 211  | W K T S A A E R F F M W I G G F R P S E L L K V L T P H L                                                       |                                                         | 238  |
| 755  | GAG CTC TTG ACA GAA CAA CAA CTT GCA GAG GTT TGT AAC CTG ACC CAA TCA TGT CAG CAA CCA GAA GAG GGC TTG TCA CAA GGA |                                                         | 838  |
| 239  | E L L T E Q Q L R E V C N L T Q S C Q Q A E D A L S Q G                                                         |                                                         | 266  |
| 839  | ATG GTA AAA CTC CAC CAG ATT CTT GCC GAG GCT GTT GCA GCT CGC GGA CTA GGA GAA GAG AAT TAC ACT CTT CCG CAG ATG GGG |                                                         | 922  |
| 267  | M V K L H Q I L A E A G V A A G A R L G G E G N Y T L P Q M G                                                   |                                                         | 294  |
| 923  | CCT GCC ATC GAA AAG TTG GAA GAT CTT GTT AGG TTC GTA AAT CAG CGC GAT CAT CTA CGA CAA GAA ACC CTC CAA CAG ATG TCC |                                                         | 1006 |
| 295  | P A I E K L E D L V R F V N Q A D H L R Q E T L Q Q M S                                                         |                                                         | 322  |
| 1007 | CGC ATC CTT AAT AGC TGC CAA GCA GCT GAG GGC TTA CTT GCG TTA GAG GAG TAC TTT GAA CGA CTT COT GTT TTA AGC TCA CAA |                                                         | 1090 |
| 323  | R I L N T C Q Q A G L L A L G E Y F E R L R V L S S Q                                                           |                                                         | 350  |
| 1091 | TGG GCT ACT CGT CTA CGT GAG CCT ACC TAA TGA AGCACAAGAGATCCGCTGTATATTACTCGAGGAGTTTGCCTTCAGAAGATGATGCTGTATTGG     |                                                         | 1190 |
| 351  | W A T R L R E P T * *                                                                                           |                                                         | 359  |
| 1191 | ACCAGAGTACTGTTCTGACTGGTATCTAAACCTATATATCAGTGGCGGAGCCACAGGTTCAAGGGCAGATGCAAAATTCAGGATTCGAATGTATTCGAATCTATTA      |                                                         | 1301 |
| 1302 | TTTGACTTATTACTGGAATTTAAACACATATATGTGATCTGAGCCAAAACCTACTAGGTTTGGATGAACCCATAAGTTATACATAGATCGGCTCCTGCTCAAGGGTGT    |                                                         | 1412 |
| 1413 | TCAGTTGAGCATCCTCTGCGGAAAATATAGTGTGTATATAAGTCAGATATTATGTGTTGAATCTTGAGCACAATTAGTGTAAATCTAGGCTTCGCCATTGAGCTATAT    |                                                         | 1523 |
| 1524 | TCATTCACTTCAGGTTGTGCGTAATGAATTTACCATCTTCTCTACTCTGTAAGGCTCTTGAGAGCTTAAATGAGATTTTACACAAATAGCC                     |                                                         | 1617 |

**b**

|     |                                                                                                                 |     |
|-----|-----------------------------------------------------------------------------------------------------------------|-----|
| 1   | GAA TTC TGT GAT TTT TCC GGA AAT CAA GCA GCT GGA GGC GTT ATG GTT ATG GAT ACT TCA TCG CCG GAG CTT CGA CAG AGC TCA | 84  |
| 1   | E F C D F S G N Q A A G G V M F M D T S S P E E L R Q S S                                                       | 28  |
| 85  | AGC GGC TCA GAT GTT TTG AAT GCA AGC TCG TCG AGC TCG TCC CAC CAG GTT TCT GGC CAT GTC GCG GGG TAC CTG AAC GTG CCA | 168 |
| 29  | S G S D V L N A T S S T S S H Q V S G D C A G Y L N V P                                                         | 56  |
| 169 | TCG CCG GAG TCC AAT GGA TCC AAC CAT GAG GGT TCT CGG GAG TCT GCT AAT GAC AAC AAG GGT TTG GGT GAT GCT AGG GTT TTG | 252 |
| 57  | S P E S N G S N H E G S R E S A N D N K G L G D A R V L                                                         | 84  |
| 253 | AAT TGC CAT TCG CCG GAG TCG CAG GGT TCA GGC AAT TAT GGT TCA AAT GTC TCA GAA GGC CTG AAT TAT CCG TCA GAT TCG AAC | 336 |
| 85  | N C H S F E S Q G S A G N Y G S N V G L N Y P S D S N                                                           | 112 |
| 337 | AAA TCG GTA CAT TCT TCT CTT AAT TTT GAA AAT AAT TCA ATA AAA AAT GGA GCT GTA GAA GAG AAA ATC AAA TTA GAG GGT GTC | 420 |
| 113 | K S V H S S P N F E N N S I K N G A V E E K I K L E G V                                                         | 140 |
| 421 | AAT GCT AAT ATA AGT AAA TGT AGC TCC TTG TTG AAG AGG AAA AAA AGT AGT GAA GAT TCT AAT AAC ATA AAC ATA CAC CAA AAA | 504 |
| 141 | N A N I S K C S S L L K R K K S S E D S N N I N I H Q K                                                         | 168 |
| 505 | TTG ACT AAT GTT GCA TTG AGT GAC AAT GTT AAT AAT GAT GAG GAT GAA AAG AAG AGA GCT AGA TTG GTT AGG AAT AGG GAA AGT | 588 |
| 169 | L T N V A L S D N V N N D E D E K K R A R L V R N R E S                                                         | 196 |
| 589 | GCT CAA CTG TCA AGG CAA AGA AAG AAG CAC TAT GTT GAG GAA TTA GAA AAT GAA GTT AGA ATA ATG CAT TCA ACA ATT CAA GAT | 672 |
| 197 | A Q L S R Q F R K K H Y V E E L E D K V R I M H S S T I Q D                                                     | 224 |
| 673 | TTG AAT GCT AAG GTA GCT TAT ATA ATT GCG GAA AAT GCT ACT CTA AAG ACG CAG                                         | 726 |
| 225 | L N A K V A Y I I A E N A T L K T Q                                                                             | 242 |

**c**

|                 |                                                            |                       |
|-----------------|------------------------------------------------------------|-----------------------|
| C-Jun (259-317) | QERIKAFKRMNRIIAADIRKRLERIAHLEKVKITLKAQNSLSTANMLREQVAGT     | Binding site sequence |
| GCN4 (223-281)  | ESDDFAALKKARNTEAARSRARLRMRQLEDKVEELLKQYHLENEVARLKLVGER     | ATGAC TCT             |
| CREB (266-324)  | EAARREVRIMKRNREAREQRKKETVKLENRMVAVLENKNTILEELKALDLYCHK     | TGAC TCA              |
|                 |                                                            | TGACGTCA              |
| TGA1a (70-128)  | KPVEKVIIRIRIRONREAAKRSRIRKKAVVQLENSKLKILQLELERARKQGMVGGGV  | TGACG                 |
| TGA1b (181-239) | DEDEKRRIRIMRNRESADISRRKKHYVERLEDKVRIMHSTIQDINAVAKIIAENATIT | TGACG                 |

FIG. 2 DNA and deduced amino acid sequences of TGA1a and TGA1b. **a**, DNA sequence of the cDNA insert in hb5. The deduced amino acid sequence for the longest ORF is shown below the DNA sequence in the single-letter code. The first methionine residue (M) in the figure, assumed to be the translation initiation site, is numbered as 1. Hb1 and -3 contained insert DNA sequences corresponding to nucleotide numbers 1-1,262 and 1-1,276, respectively. **b**, DNA sequence of the cDNA insert of hb2. The deduced amino acid sequence is shown as **a**. The first glutamate residue (E) in the insert is numbered as 1 for convenience. Hb6 contained insert DNA sequence very closely related to that of hb2 nucleotide numbers 1-676 (~95% homology). **c**, Amino-acid residues 70-128 of TGA1a and amino-acid residues 181-239 of TGA1b are compared with homologous regions of c-Jun, GCN4, and CREB<sup>1-4</sup>. The positions of the homologous regions are indicated in parentheses as amino-acid residue numbers. The binding-site sequences for the five DNA-binding proteins are also indicated. Conserved residues between TGA1a, TGA1b, and the other three proteins are enclosed by thin lines, whereas the residues conserved only among TGA1a, TGA1b, and CREB are enclosed with bold lines. Asterisks indicate leucine residues for the leucine-zipper.

**METHODS.** The cDNA inserts of hb1, -2, -3 and -5 were subcloned as restriction fragments into M13mp18 and M13mp19 vectors. Nucleotide sequences of both strands were determined by Sequenase<sup>TM</sup> sequencing kit (USB) with common primers and synthesized primers. To obtain overlaps between some subclones, double-stranded DNA sequencing was carried out using the pUC sequencing kit (Boehringer). Sequence data were processed by DNASIS and PROSIS programs (Hitachi) on an IBM PS/2 computer. The homologies shown in **c** were detected by visual inspection.



a basic region (amino-acid residues 70–94; 11 basic and 2 acidic), a putative leucine-zipper region<sup>5</sup> (leucine residues 100, 107, and 114), and a relatively glutamine-rich region (amino-acid residues 244–332; 14 glutamine residues). The acidic region could be involved in activation of transcription, because acidic domains function as activating regions in several DNA-binding proteins<sup>9</sup>.

Figure 2b shows the DNA sequence of the insert of hb2, which encodes TGA1b, and the deduced amino acid sequence for the longest ORF. The ORF is in-frame with the reading frame of *lacZ*. Although this was not a full-length clone, the deduced polypeptide has some interesting characteristics. It contains a serine-rich region (amino-acid residues 20–160; 33 serine residues), a basic region (residues 185–206; 11 basic residues and 1 acidic residue), and a putative leucine-zipper region (leucine, methionine, or isoleucine residues at positions 211, 218, 225, 232, and 239). There is no significant homology between TGA1a and TGA1b other than the basic region and the leucine-zipper region.

The basic regions of TGA1a and TGA1b are homologous to those in the DNA-binding domains of CREB, GCN4, and

c-Jun<sup>1–4</sup> (Fig. 2c). The binding site of CREB contains a TGACG motif, whereas the binding sites of GCN4 and c-Jun contain a similar TGACT motif<sup>1–4</sup>. Among the three, CREB has the highest homology to the basic region in TGA1a and TGA1b. It seems reasonable to predict that the amino-acid residues that are conserved only among CREB, TGA1a, and TGA1b (enclosed by bold lines in Fig. 2c) are important in distinguishing the TGACG motif from the TGACT motif. This prediction could be tested by site-directed mutagenesis. The nuclear factors CREB, GCN4, and c-Jun are members of a class of DNA binding proteins that contain a leucine-zipper region<sup>6</sup>. This region is at the carboxy-terminal end of the basic region. When the amino-acid sequences of these proteins are aligned according to their homology in the basic region, the first three of the four leucine residues (marked by asterisks in Fig. 2c) of the leucine zipper in CREB, GCN4, and c-Jun are conserved in TGA1a. In TGA1b, residues in the corresponding positions are Leu, Met, Leu, Ile and Leu. Because a leucine residue in the leucine-zipper of the heat-stable DNA-binding protein C/EBP can be substituted by a methionine or isoleucine residue (although with some reduction in the potential of the protein to form dimers)<sup>11</sup>, we consider this region of TGA1b to be a variant of the leucine-zipper. Such a high degree of conservation among these proteins that come from distantly related organisms (human, yeast, and higher plant), suggest that these leucine residues are functionally equivalent.

Northern blot analysis of tobacco RNA with the TGA1a cDNA insert showed that the level of TGA1a mRNA (1.8 kb) in roots was ~10 times higher than it was in light-grown or dark-adapted leaves (Fig. 3a). The level of TGA1b mRNA (2.9 kb) in roots was two- to fivefold higher than it was in leaves; dark-adapted leaves contained twice as much TGA1b mRNA as light-grown ones (Fig. 3a). As a control, we found that the expression of the beta subunit of mitochondrial ATP synthase (b-ATPase) was about twofold higher in roots than it was in leaves, confirming previous results (Fig. 3a)<sup>11</sup>. The finding of much higher expression of TGA1a in roots than in leaves is correlated with the observation that *as-1* is involved in the root expression of the 35S promoter<sup>13</sup> and is consistent with the proposal that the functional form of ASF-1 could be limiting in leaves.

Southern blot analyses of the genes encoding TGA1a and TGA1b showed only a few bands under relatively stringent conditions of hybridization (Fig. 3b). Under these conditions, the two genes did not cross-hybridize. Thus, it is likely that each of them is encoded by a small gene family. Because northern blot analyses gave only a single band for each gene (Fig. 3a), both gene families probably contain very closely related members.

The high homology in the DNA-binding domains among TGA1a, TGA1b, and CREB suggests that they have an ancient origin, because plants and animals diverged  $\sim 1.5 \times 10^9$  years ago<sup>12</sup>. Analysis of the TGA1a and TGA1b genomic clones will reveal whether this DNA-binding domain is encoded by a separate exon. Moreover, the extent of homology suggests possible conservation in functions other than their DNA binding specificity. In mammalian systems, CREB is involved in signal transduction pathways through kinases, notably protein kinase A. The proteins TGA1a and TGA1b may be involved in similar signal transduction pathways in higher plants. □

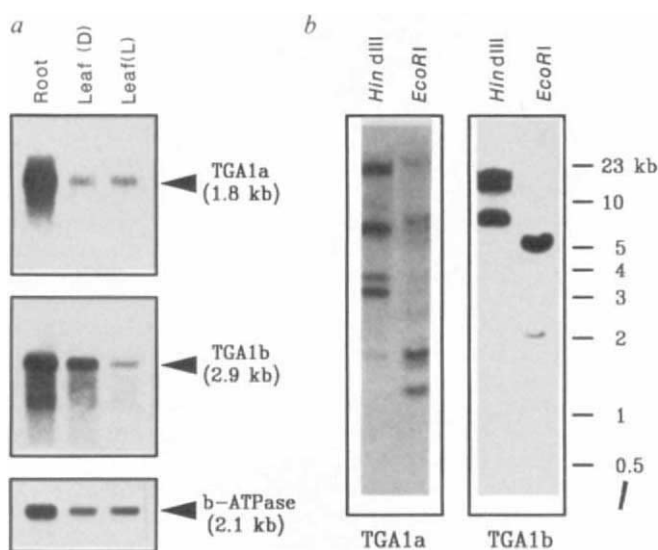


FIG. 3 Northern and Southern blot analyses of TGA1a and TGA1b genes. *a*, Northern blot analysis of RNA from tobacco leaves and roots with TGA1a and TGA1b genes. Each lane contained 2  $\mu$ g of poly(A)<sup>+</sup> RNA. Messenger RNA sizes were determined by comparison with molecular size markers. Leaf(D), leaves from tobacco plants kept in the dark for two days; Leaf(L), leaves from light-grown tobacco plants; Root, roots from light-grown tobacco plants. *b*, Southern blot analysis of TGA1a and TGA1b. Each lane contained 17  $\mu$ g of tobacco DNA digested with either *Hind*III or *Eco*RI. Positions of molecular size markers are indicated on the right.

**METHODS.** Preparation of RNA and DNA samples from *Nicotiana tabacum* cv. SR1 and northern and Southern blot analysis were as described.<sup>8</sup> The 1,150-bp *Eco*RI-*Xba*I fragment from hb1, the 560-bp *Eco*RI-*Fok*I fragment from hb2, and the 1.3-kb *Hind*III-*Xba*I fragment from the b-ATPase gene *atp2-1* (ref. 11) were labelled by random primed DNA labelling kit (Boehringer) and used as the TGA1a, the TGA1b, and the b-ATPase probe, respectively. The specific activities of the probes were  $\sim 1.8 \times 10^9$  c.p.m. per  $\mu$ g. The same filter was sequentially hybridized with TGA1a, TGA1b, and b-ATPase probes, after washing off the previous probe by boiling in water. Filters were pre-hybridized and hybridized in a buffer containing 50% formamide, 10% dextran sulphate, 25  $\mu$ g ml<sup>-1</sup> sonicated and denatured salmon testis DNA, 1  $\times$  Denhardt's solution, and 5  $\times$  SSC at 43  $^{\circ}$ C. About  $4 \times 10^6$  c.p.m. ml<sup>-1</sup> of probes were used. The filters were washed in 2  $\times$  SSC, 0.1% SDS and then in 0.1  $\times$  SSC, 0.1% SDS at room temperature. They were washed finally in 0.1  $\times$  SSC, 0.1% SDS at 50  $^{\circ}$ C, briefly dried, and then autoradiographed with an intensifying screen. The exposure times for TGA1a, TGA1b, and b-ATPase in *a* were 3 h, 1 day, and 1 h, respectively. The exposure times for TGA1a and TGA1b in *b* were 7 h and 10 h, respectively.

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## Three-dimensional structure of *Escherichia coli* RNA polymerase holoenzyme determined by electron crystallography

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**DURING transcription in *E. coli*, the DNA-dependent RNA polymerase locates specific promoter sequences in the DNA template, melts a small region containing the transcription start site, initiates RNA synthesis, processively elongates the transcript, and finally terminates and releases the RNA product. Each step is regulated by interactions between the polymerase, the DNA, the nascent RNA, and a variety of regulatory proteins and ligands<sup>1–3</sup>. The *E. coli* enzyme contains a catalytic core of two  $\alpha$ -subunits, one  $\beta$ - and one  $\beta'$ -subunit, with relative molecular masses ( $M_r$ ) of 36,512, 150,619 and 155,162, respectively<sup>2</sup>. The holoenzyme has an additional regulatory subunit, normally  $\sigma_{70}$ , of  $M_r$  70,236. Preparations may also contain the  $\omega$ -subunit ( $M_r$  ~10,000), which can be removed without affecting any known properties of the enzyme<sup>2</sup>. Because the amino-acid sequences of the  $\beta$ - and  $\beta'$ -subunits are homologous to those of the largest subunits of the yeast, *Drosophila* and murine RNA polymerases<sup>4–7</sup>, it seems likely that essential features of the three-dimensional structure and catalytic mechanism of RNA polymerase are also conserved across species. Crystals of RNA polymerase suitable for X-ray analysis have not yet been obtained, but two-dimensional crystals of *E. coli* RNA polymerase holoenzyme can be grown on positively charged lipid layers<sup>8</sup>. Electron microscopy of these crystals in negative stain shows the enzyme in projection as an irregularly shaped complex ~100 × 100 × 160 Å in size. We have now determined the three-dimensional structure by electron microscopy of negatively stained, two-dimensional crystals tilted at various angles to the incident electron beam<sup>9</sup>. We find a structure in RNA polymerase similar to the active-site cleft of DNA polymerase I (ref. 10). In the light of functional similarities between these two enzymes, together with other evidence, this probably identifies the active-site region of RNA polymerase.**

Two-dimensional crystals of *E. coli* RNA polymerase holoenzyme were formed on layers of positively charged lipid, transferred to an electron microscope grid, and negatively stained with 1% uranyl acetate as described previously<sup>8</sup>. The crystals contained holoenzyme, and not simply core enzyme that had lost the  $\sigma_{70}$  subunit during the crystallization process, as monoclonal antibodies to the  $\sigma_{70}$  subunit<sup>11</sup> bound specifically to the crystallized RNA polymerase (shown by electron microscopy; S.A.D., N. E. Thompson and R. R. Burgess, data not shown). Furthermore, purified core enzyme failed to crystallize under the same conditions. Presumably, the crystals also contained the  $\omega$ -subunit, as this polypeptide was present in roughly stoichiometric amounts in the sample used for crystallization (D. N. Arnosti and M. J. Chamberlin, personal communication). Crystalline areas ranged in size from 0.2 to 0.7  $\mu\text{m}^2$  and were

~100 Å thick<sup>8</sup>. The array can be described as a crystal of space group P2 ( $a = 201$  Å,  $b = 200$  Å,  $\gamma = 107^\circ$ ) with a thickness of one unit cell in the direction of the  $c$  axis. Thus, each crystalline area contained 500–2,000 unit cells or 1,000–4,000 molecules.

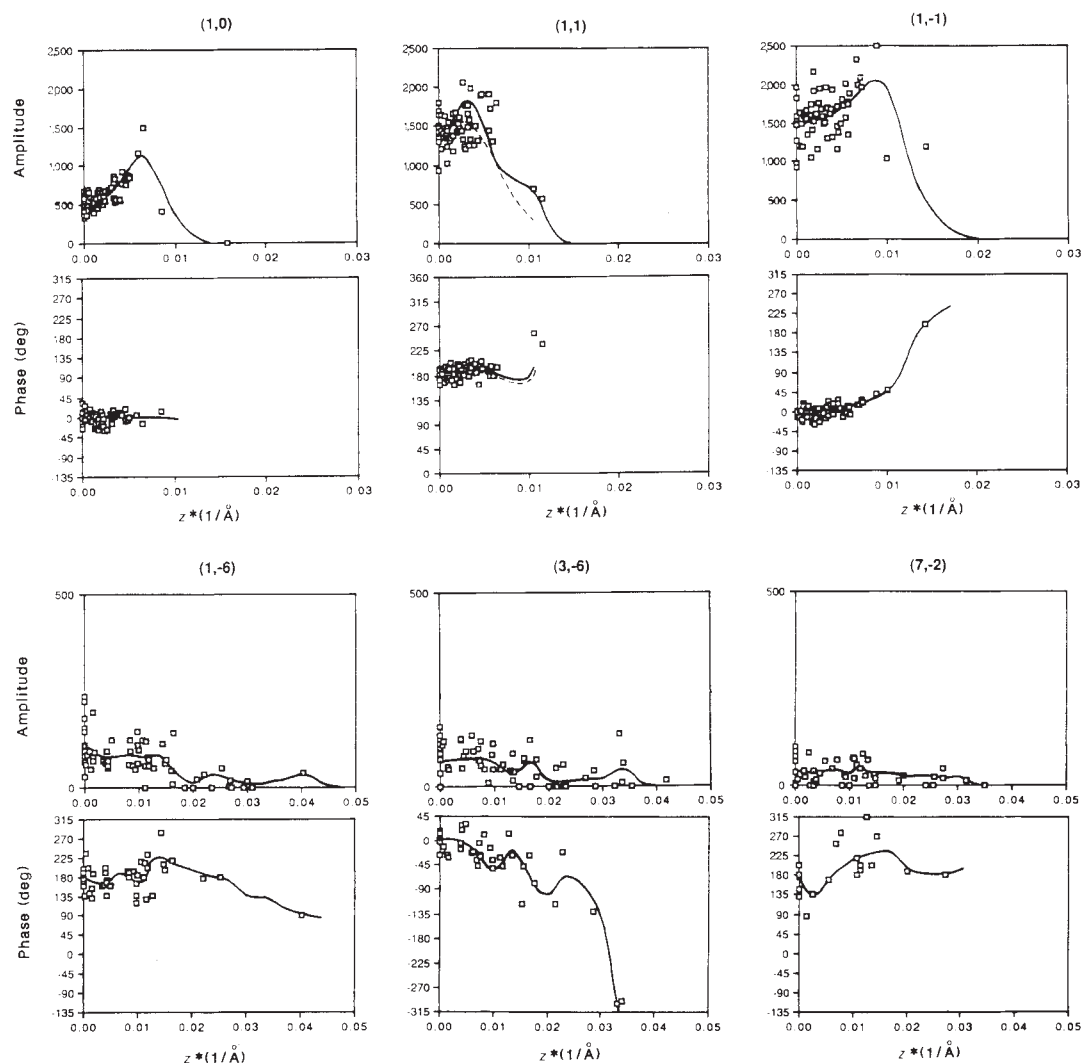
Three micrographs were recorded from each crystalline area using low electron doses (<10 electrons per Å<sup>2</sup>) at a calibrated magnification of ×27,800, two with the specimen tilted to the incident 100 kV electron beam, and one untilted. Micrographs of test areas showed no noticeable degradation in the diffraction data after three exposures, even for the highest resolution spots observed. Micrographs of similar quality were selected, digitized, and processed by computer using standard methods<sup>9</sup>. Additional computations incorporated procedures for correcting lattice distortions by reciprocal space filtering, real space correlation analysis, and spline function interpolation, as described by Henderson *et al.*<sup>12</sup>.

A total of 74 micrographs of crystals tilted 0–80° to the incident electron beam were used. The average phase error, based on refinement of each new measurement with all previously accumulated symmetry-related phases within 0.00227 Å<sup>−1</sup> in  $z^*$ , was 14° when the data were combined according to the crystallographic space group P2. No single micrograph had an average phase error greater than 20°. Refinements were also performed to investigate the possibility of 2-fold or screw axes in the plane of the lattice. The minimum average phase error was 39° in each case. A refinement of the data was also performed assuming no symmetry (space group P1). The resulting structure showed two regions of protein density per unit cell, both very similar in appearance to each other and to the final structure determined assuming P2 symmetry, and strongly related by a 2-fold axis perpendicular to the lipid layer. No other symmetry elements were apparent. These results and the excellent refinement of the data assuming P2 symmetry strongly supported the choice of space group. Finally, either molecule in the unit cell of the P1 structure was consistent with the conclusions of this work.

A least-squares procedure<sup>13</sup> was used to fit smooth curves (Fig. 1) to the combined amplitude and phase data for each of the 93 crystallographically independent lattice lines included in the reconstruction. By sampling these curves at intervals of 0.00333 Å<sup>−1</sup> in  $z^*$ , 552 Fourier terms were collected and used to calculate the three-dimensional map. Except for the cone of reciprocal space not sampled because of the upper limit on tilt angles, the data were nearly complete to a resolution of ~27 Å, with a considerable amount of information beyond that resolution (Fig. 2). To help assess the effect of the missing data, edge-on views of the crystals<sup>8</sup> were analysed to give directly the variations in amplitude and phase along the 1, 1 lattice line. Estimates of the variations along the 0, 0 lattice line were also obtained from the edge-on views, but this information was obscured by noise near the origin of the transforms because of uneven stain distribution across the lipid layer. The data from edge-on views for the 1, 1 lattice line were adjusted to give the best fit to the 1, 1 data from the tilt experiments (Fig. 1). The amplitude scale factor and phase origin adjustment needed to fit the 1, 1 data were used to correct the estimated variations along the 0, 0 lattice line. It was found that the basic features of the three-dimensional map were not affected by including estimates of amplitude and phase along the 0, 0 lattice line. Thus, owing to the poor quality of the 0, 0 lattice line data, they were not included in the final reconstruction presented here.

The map calculated for RNA polymerase in negative stain shows the surface topography of the molecule, so the outermost contour is of interest. This contour was chosen such that the individual molecules in the array were completely disconnected. The overall dimensions of a protein molecule were then ~90 × 95 × 160 Å, consistent with the dimensions determined previously by analysis in projection and from views of single particles<sup>8,14</sup>. Assuming a protein density of 1.3 g cm<sup>3</sup>, the volume occupied by a molecule corresponds to an  $M_r$  ~370,000, about 82% of that expected for the RNA polymerase holoenzyme

FIG. 1 Values of amplitude and phase determined along reciprocal lattice lines from micrographs of specimens tilted to the incident electron beam at angles 0–80°.  $z^*$  is the distance in reciprocal space in the direction of the  $c^*$  axis. The tilt range 0–55° was obtained using a goniometer stage; additional tilts up to 80° were obtained by bending the specimen grids. The data have been combined, assuming the space group P2. The smooth curves (solid lines) drawn through the data were determined by a least-squares procedure<sup>13</sup>. Only phase data from reflections with amplitudes greater than twice background were plotted and used in the curve fitting. Shown are three of the strongest amplitude lattice lines (1,0; 1,1; 1, -1) and three lattice lines near the resolution limit of ~27 Å (1, -6; 3, -6; 7, -2). Shown along with the data for the 1,1 lattice line are the data obtained from edge-on views (broken lines), which were adjusted to give the best fit to the tilt data.



(~450,000)<sup>2</sup>. The small discrepancy is probably mainly due to some penetration of the RNA polymerase molecule by negative stain, which is excluded from hydrophobic regions but not necessarily by the outer surfaces of proteins<sup>9</sup>.

The most striking feature of the map is a thumb-like projection that surrounds a cylindrical channel. Viewing the map parallel to the plane of the lipid layer (Fig. 3a), one looks towards the front of the channel, whereas viewing the map at an angle from beneath the lipid layer (Fig. 3b), one sights along the axis of the channel. The channel is ~25 Å in diameter and 55 Å in length, and is thus of appropriate dimensions for binding double-helical DNA. Support for this possible role comes from a remarkable similarity between the channel and the proposed DNA-binding-site cleft in the X-ray crystal structure of the large (Klenow) fragment of *E. coli* DNA polymerase I (ref. 10). Figure 3 illustrates the good fit of size and shape in the region of the cleft between the RNA polymerase map and the  $\alpha$ -carbon backbone of DNA polymerase. An apparent deviation occurs at the tip of the thumb-like projection of the RNA polymerase map, where there is a region of density not matched by any structural features in the DNA polymerase  $\alpha$ -carbon backbone. At just this point in the DNA polymerase structure, however, there is a stretch of 50 residues that are not resolved because of disorder or motion in the crystal. The  $\alpha$ -carbon atoms on each side of the unobserved gap are indicated in red (Fig. 3). The position of the extra density in the RNA polymerase map is clearly consistent with it being the structural counterpart of

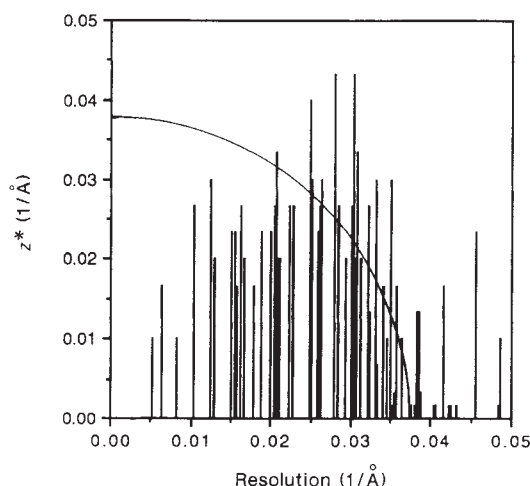


FIG. 2 Schematic diagram showing the region of reciprocal space used in the reconstruction. The 27-Å resolution sphere is included.



the flexible domain in the DNA polymerase structure. The low resolution of the RNA polymerase structure (compared with that of the X-ray structure of DNA polymerase) allows this feature to be observed. The existence in DNA and RNA polymerases of this flexible domain, which might surround the DNA substrate, suggests a means by which these enzymes could act processively in the polymerization of their respective products<sup>10,15</sup>.

The significance of the structural similarity between DNA and RNA polymerases is supported by a small degree of amino-acid sequence conservation<sup>4</sup>. There is a weak homology between residues 666–695 in DNA polymerase I and residues 350–380 of the  $\beta$ -subunit of RNA polymerase<sup>4</sup>. The conserved residues are on the floor of the DNA-binding cleft of DNA polymerase I (ref. 10), part of the region that matches well with the RNA polymerase structure. Moreover, the  $\beta'$ -subunit of RNA polymerase is believed, based on other grounds, to be involved in DNA binding<sup>1</sup>.

The 55 Å length of the putative DNA-binding channel in the RNA polymerase map is sufficient to accommodate ~16 base pairs of double-helical DNA in the B form, which may relate to the 16–18 base pairs that become unwound in the transcription complex<sup>16</sup>. This single-stranded region lies within a longer

stretch of 50–60 base pairs (at least 170-Å long in the B form) associated with the enzyme in the open-promoter complex<sup>17</sup>. There does not seem to be any way in which a 170 Å length of DNA can be bound to the enzyme without significant bending of the DNA. Evidence for such bending has come from electrophoretic-mobility shift experiments<sup>18</sup>. The path followed by the DNA across the surface of the enzyme remains to be seen. Crystallization and imaging of enzyme–DNA complexes by the methods used here should help assess the role of the putative DNA-binding channel and reveal other features of the protein–DNA interaction. □

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## ERRATA

### New light on the Lysenko era

Valery N. Soyfer

*Nature* **339**, 415–420 (1989)

IN this Commentary, the author's affiliation (page 420) should read: Department of Molecular Genetics and Biotechnology Center, The Ohio State University, Columbus, Ohio 43210, USA. And the sentence running from page 417 to page 418 should read: "Lysenko had also harmed his own cause by his resolute repudiation of plant hormones, and his reputation was undermined when the Dutchman Went and the Soviet psychologist N. G. Kholodony were honoured for their discovery." □

### Substrate specificity and affinity of a protein modulated by bound water molecules

F. A. Quiocho, D. K. Wilson & N. K. Vyas

*Nature* **340**, 404–407 (1989)

THE 'Acknowledgements' section was omitted from the above letter during the production process. It should read: This work was supported by the Howard Hughes Medical Institute and grants from NIH and the Welch Foundation. □

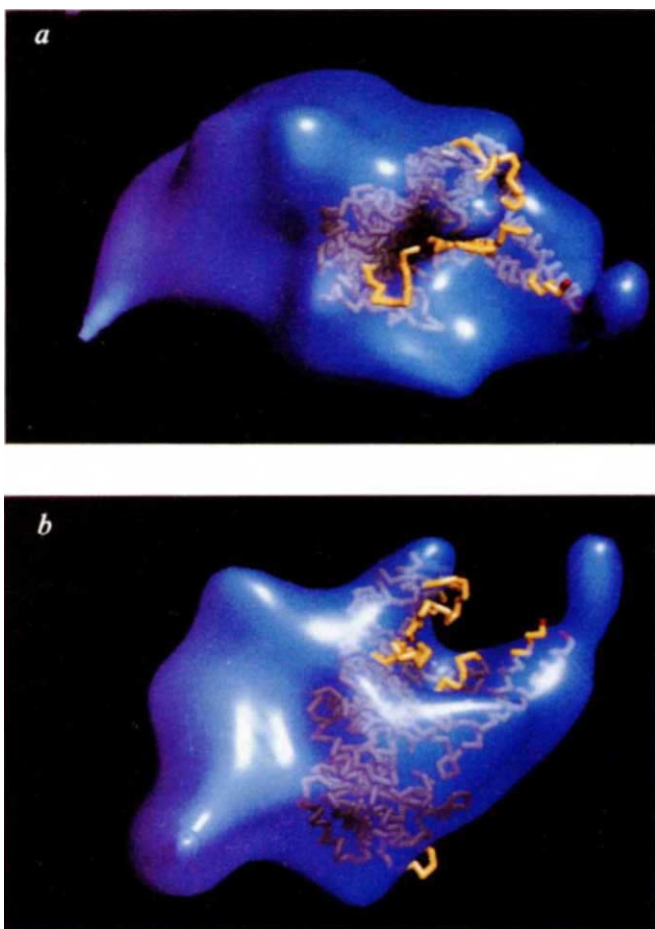


FIG. 3 Computer-generated models of a single *E. coli* RNA polymerase holoenzyme molecule. Matched to the RNA polymerase map is the  $\alpha$ -carbon backbone of the DNA polymerase I Klenow fragment<sup>10</sup> (shown in grey, except for parts protruding from the RNA polymerase map, which are shown in yellow). The  $\alpha$ -carbon atoms on each side of the unobserved gap in the DNA polymerase chain<sup>10</sup> are shown in red. The contour represents negative stain-excluding region. *a*, The molecule viewed in the plane of the two-dimensional array and perpendicular to the crystallographic *a*-axis. The lipid layer to which the molecule is adsorbed would lie underneath. *b*, The molecule viewed from the bottom (as if looking up through the lipid layer) along the axis of the cleft. Images courtesy of D. S. Goodsell and A. J. Olson<sup>19</sup> (magnification,  $\times 4.7 \times 10^6$ ).

# Magnetic separation of DNA

M. Uhlen

Originally developed for immunoassays, magnetic beads in combination with streptavidin-biotin technology have demonstrated their power for separating DNA and RNA.

SOLID-phase methods have proven to be very useful for the separation, synthesis and diagnostic detection of biomolecules. The solid-phase approach produces reproducible reactions with high yields, and allows solutions to be changed rapidly. Routine methods suitable for automation can be designed relatively easily using a solid phase, because of the ease with which it can be separated from the reaction solution.

The solid phase may consist of the walls of test tubes or microtitre wells, or polymer particles (such as agarose or silica) packed in columns. Magnetic particles can also be used as a solid support for the separation of non-clarified cell suspensions or lysates without the need for centrifugation or filtration. If the magnetic beads are non-porous, adsorption and desorption of biomolecules occur at the surface, providing reaction kinetics similar to those found in free solution.

## Attractive separations

Early magnetic particles, made by the polymerization of acrylamide and agarose with paramagnetic materials<sup>1</sup>, were heterogeneous in size and magnetite content. Hydrophilic beads have now been developed that are identical in size, density and amount of magnetized material<sup>2</sup>. Such beads sediment homogeneously in magnetic fields, and separations using them can often be accomplished within a few seconds. The chemical structure of the particle surface may be varied<sup>3</sup>, providing a flexible system for the immobilization of biomolecules.

Immunomagnetic separations, using immobilized monoclonal and polyclonal antibodies, have been described where the magnetic beads are directed to targets such as cells, organelles or macromolecules. The speed of magnetic separation is thus combined with the specificity of antibodies. Immunological applications based on this concept have been developed for tissue typing and cancer therapy, as well as for the selection of monoclonal antibody-producing cells and the separation of sub-cellular components<sup>4</sup>.

## Biotin-streptavidin

Magnetic separation can also be used as a tool in molecular biology, by coupling specific DNA fragments to the beads and using them as probes or templates. The fragments can be bound to the beads through a reactive amino or thiol group on

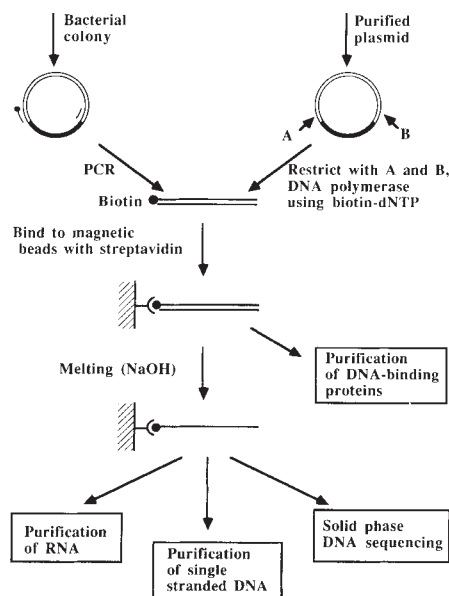


FIG. 1 Possible applications for DNA-containing magnetic beads in molecular biology.

a synthesized oligonucleotide, using the procedures developed for the immobilization of antibodies. But a more versatile system uses magnetic particles covalently bound to streptavidin, for the directed immobilization of both double-stranded and single-stranded biotinylated DNA. The remarkable stability and strength ( $K_d = 10^{-15}$ ) of the non-covalent biotin-streptavidin interaction allows DNA manipulation reactions, such as strand melting, elution and hybridization (using alkali, temperature or formamide), to be performed without interfering with the binding of the DNA to the beads<sup>5</sup>.

The DNA-bead complex is also resistant to high concentrations of urea and salt. Several alternative strategies exist for the selective introduction of biotin into one of the strands of the DNA. The most straightforward approach is to synthesize an oligonucleotide containing a chemically introduced biotin molecule<sup>6</sup>, and then directly immobilizing the oligonucleotide onto the streptavidin-coated bead. An even more flexible approach (Fig. 1) is to perform endonuclease restriction of a purified plasmid, followed by a fill-in reaction with DNA polymerase using one or several biotinylated nucleotides<sup>5</sup>. Alternatively, a polymerase chain reaction (PCR)<sup>7</sup> can be performed using thermostable DNA polymerases such as *Taq* or *Tth* and a biotinylated oligonucleotide<sup>8</sup>. The latter two procedures can

immobilize cloned fragments in sizes ranging from a few nucleotides to several kilobases. The techniques also yield immobilized double-stranded DNA, which may be converted into single-stranded DNA through an appropriate melting procedure<sup>5,8</sup>.

## Molecular applications

The use of magnetic beads in combination with the biotin-streptavidin system has allowed the direct solid-phase sequencing of both genomic and plasmid DNA<sup>8</sup>. The system allows the *in vitro* amplification and sequencing reactions to be performed under optimal conditions because the buffers and enzymes can be changed easily. A protocol has been described for the direct sequencing of plasmid DNA starting from a single bacterial colony, without the need for previous template purification<sup>8</sup>.

The rapid magnetic system can also be substituted for conventional affinity chromatography for the purification of DNA-binding proteins. Three cycles of adsorption and desorption to magnetic beads containing a specific double-stranded recognition sequence has yielded a nearly homogeneous nuclear factor (IIIC) in less than one hour from a yeast crude extract<sup>9</sup>.

Beads containing single-stranded DNA can be used for the separation and isolation of RNA and single-stranded DNA. Using monosized beads with coupled oligo(dT)<sub>30</sub>, mRNA has been purified with a high yield from a crude extract of a mouse hybridoma cell line within two minutes<sup>10</sup>. The low number of RNA handling steps and the short time required to perform the technique reduce the risk of physical and enzymatic degradation of the RNA. It is also possible to isolate specific RNA molecules using cloned or synthesized DNA fragments as probes. Obviously, the RNA molecules recovered on the magnetic beads can subsequently be used for solid-phase cDNA synthesis.

The magnetic particles can also be used to recover specific single-stranded DNA fragments from a complex mixture of fragments<sup>11</sup>. New cloning strategies can be envisioned where DNA fragments obtained from plasmid, lambda, cosmid or genomic DNA could be specifically recovered by the magnetic beads and subsequently amplified using PCR. In this way, chromosome walking and other operations could be achieved without the need for subcloning.



Finally, the magnetic solid-phase technology can be used for separation steps during the analysis of specific DNA or RNA sequences. Such an assay has been described which uses a DNA probe-based hybridization reaction for the detection of HIV-1-specific RNA in infected cells<sup>12</sup>. Specific hybridization complexes can be purified using a magnetic approach called target cycling/background reduction, in which hybrids are captured from solution with a complementary sequence attached to magnetic particles. We have recently used magnetic beads in an assay for the detection of immobilized amplified nucleic acids, in which samples amplified using a two-step PCR are directly captured using the biotin-streptavidin system. The amount of immobilized amplified fragment can then be detected using an isotope or colourimetric procedure. The assay is rapid and might — in some applications — eliminate the need for electrophoresis, restriction mapping or hybridization assays in the analysis of PCR-amplified materials. □

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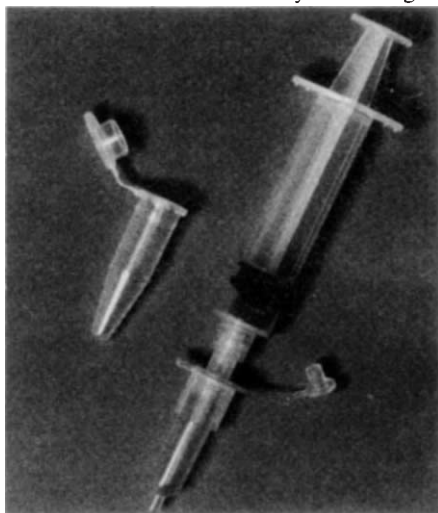
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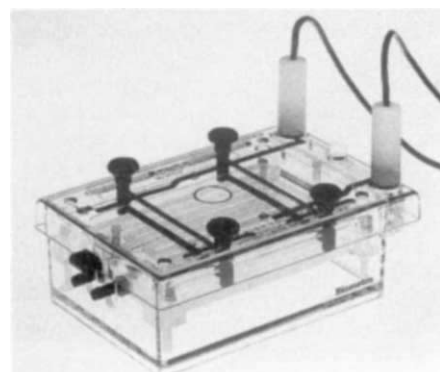
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Mini-Focus is the name of the new gel apparatus from Biometra for **flat bed isoelectric focusing** using either polyacrylamide or agarose gels (*Reader Service No. 104*). The electrodes — made of synthetic carbon — are placed in direct contact with the gel surface, eliminating the need for extra buffers and wicks. Biometra says this arrangement produces a uniform electric field and results in sharp bands and high reproducibility. The £645 (UK) gel



Mini-Focus — new electrophoresis hardware for isoelectric focusing from Biometra.

apparatus comes complete with condensation shield, height-adjustable cooling device and electrodes. The gel chamber can accommodate either analytical or preparative gels on gel bond foils that

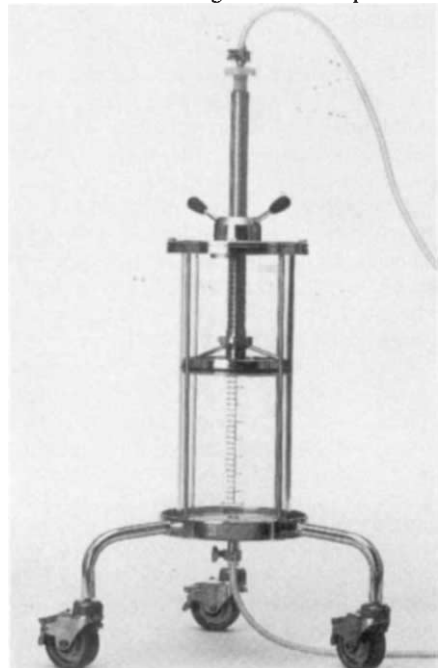


are 0.1–10-mm thick and up to 10 cm in length. Mini-Focus can be used in conjunction with Biometra's Minigel apparatus for 2-D electrophoresis.

International Biotechnologies, Inc. has a programmable **polarity switching system** for the separation of large DNA molecules (*Reader Service No. 105*). The Fiji HV system comes in two models: the \$2,450 (US) HV600 and the \$2,950 (US) HV3000, which have voltage ranges of 0–700 V and 0–3,000 V, respectively. The two-component system consists of a switching device that can control up to four gels simultaneously, and a remote keypad. The system automatically ramps time intervals and can switch polarity at intervals as short as 0.001 seconds, says IBI. Operating parameters are entered using the menu-driven programming interface, which can store up to 99 switching protocols. IBI says the system has applications in megabase gene mapping, gene linkage studies, and in restriction fragment mapping of lambda, cosmid and yeast clones.

### Chromatography columns

The process-scale purification of proteins, peptides or other biomolecules need not be a problem with the new range of **glass chromatography columns** from Pharmacia LKB (*Reader Service No. 106*). BioProcess Glass Columns have a 3-bar operating pressure and are available with diameters of 100 or 200 mm and lengths of 500, 750 or 950 mm. The single-screw adaptor can

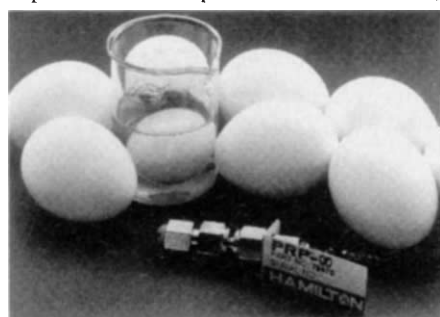


Glass chromatography column for process-scale protein purification from Pharmacia.

be adjusted to give a wide range of bed heights and bed volumes and makes even contact with the gel bed. Designed for hygienic operation and maintenance, the column is made of borosilicate glass and

electropolished steel, which Pharmacia says resists corrosion, reduces friction and hinders microbial attachment. Column packing is a simple procedure as the gel bed is clearly visible through the graduated glass tube. The column can be used for many separation techniques, including desalting/buffer exchange, ion exchange, affinity chromatography, hydrophobic interaction and gel filtration.

Hamilton Co. says its new polymeric **reversed phase HPLC column** can resolve high molecular weight proteins that differ by only a few amino acids (*Reader Service No. 107*). The \$290 (US) non-porous PRP- $\infty$  column can produce gradient separations of six proteins in 60 seconds,



Hamilton's non-porous PRP- $\infty$  column for high-speed protein separations.

keeping peaks narrow for improved detectability and high resolution, says the company. The 30  $\times$  4.1-mm column is packed with a polystyrene-divinyl benzene material that is stable up to pressures of 5,000 p.s.i. The stability of the column from pH 1 to 13 allows samples to be analysed at basic pH (8–13), which Hamilton says improves detectability and can provide confirmation of protein purity.

The GS Series of Asahipak GPC columns from Advanced Separation Technologies, Inc. have a packing material based on **hard gel polymers** high in hydroxyl content, and can be used for HPLC of both hydrophilic and hydrophobic substances (*Reader Service No. 108*). Using the Asahipak GPC columns, dual mode chromatography can be performed, which effectively combines both gel permeation chromatography and adsorption chromatography in the same column. The matrix can withstand high pressures and high flow rates, and is stable up to pH 14, says the company. A wide range of organic solvents can be used with GPC columns, says ASTEC, without the bed shrinkage associated with conventional packing materials. Asahipak columns can be used for the separation of proteins, enzymes, nucleic acids, peptides, vitamins, polysaccharides and oligosaccharides, sugars, and drugs and metabolites in body fluids. The columns are available in analytical and preparative sizes and prices range from \$800 to \$6,150 (US).

A new line of **multi-dimensional polymeric columns** that combine the power of ion exchange, reversed-phase separation and ion pairing into a single column are available from Dionex (*Reader Service No. 109*). Users can elute both inorganic and organic analytes in the same run, attaining a selectivity that Dionex says cannot be achieved using single mode separation analysis. The first two columns from the OmniPac line are the OmniPac PAX-500 and PAX-100. These 4  $\times$  250-mm columns have identical ion-exchange properties, but the PAX-100 has lower reversed-phase characteristics. The ion exchange sites are very hydrophilic and have a high affinity for hydroxide anions. Dionex says that due to the excellent bed stability of Omnipac columns, they are 100 per cent solvent-compatible and stable over the entire pH range of 0–14. Dionex will introduce columns with cation exchange and reversed-phase properties in the autumn.

### Centrifugal separation

A microprocessor-controlled **high-speed microcentrifuge** that automatically compensates for unbalanced tubes and converts r.p.m. to g-values is available from Camlab Ltd (*Reader Service No. 110*).

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Reader Service No.15

The model 157 has an operating speed spanning 100 to 30,000 g. The centrifuge recognizes the code of the rotor in use and ensures that the g-value setting is correct and does not exceed the maximum permissible value. Up to 99 programs can be entered and stored, and a large 80-character LCD provides the user with details of the preprogrammed values. The model 157 is available with and without refrigeration, for £3,790 (UK) and £2,490 (UK), respectively. Camlab offers 30 types of rotor for use with the centrifuge, each costing between £100–300 (UK).

Alfa-Laval Sharples Ltd says its Centritech Cell is a new type of separation system for **concentrating mammalian and other cell types** that are sensitive to shear forces (*Reader Service No. 111*). The company says the system has been used successfully to concentrate feline leukaemia cells from 2.58 to 36.7 million cells per millilitre, with 99.9 per cent cell viability after separation. The completely enclosed system offers no risk of contamination of cells or media and no risk to the user from harmful products. The company



Alfa-Laval's Centritech Cell, separation for shear-sensitive cells.

says the machine provides gentle acceleration and exerts the minimum centrifugal force required to effect separation. The system offers continuous separation, and at preprogrammed intervals the cell concentrate and clarified media are automatically discharged from a disposable bladder.

Beckman Instruments, Inc. has a new class of **ultracentrifuge** featuring a drive mechanism that is so stress-tolerant that rotor loads can be balanced by eye (*Reader Service No. 112*). The \$31,500–36,500 (US) L and \$37,000–42,000 (US) LX Optima Series operate without CFCs or liquid coolants over a temperature range of 0–40 °C. The XL models, which have menu-driven operation, provide automatic rotor logging, run data recording, and storage of ten run programs with up to five speed changes per run. The XL's calculation function can simplify and speed up run conversions by performing many of the calculations necessary for

CsCl and sucrose gradients. The XL's Efficient Sedimentation Program automatically adjusts speed and time to provide efficient run times without gradient precipitation, and provides run simulations that forecast the condition of the gradient and separation components. The L Series has many of the control features of the XL Series, including the ability to store programs.

### Modular HPLC systems

Perkin-Elmer has introduced a gradient LC system for high-resolution **peptide mapping** (*Reader Service No. 113*). The modular system comprises an injector, printer/plotter, Vydac C18 analytical column, scavenger column for cleaning out contaminants in the mobile phase, LC-135 dual channel detector, and model 250 Binary Pumps. The \$19,600 (US) system is configured specifically for high-sensitivity peptide mapping, which Perkin-Elmer says has a 5–10 pmol sensitivity and provides the flow rate precision necessary to reproduce peptide maps exactly.

Hitachi announces its new line of **components and systems for HPLC** that can be configured together to form a stand-alone system with the flexibility of a modular design (*Reader Service No. 114*). Hitachi offers isocratic and gradient pumping systems made of both stainless steel and inert components and costing between \$4,495–10,680 (US). The \$4,495 (US) model 4200 variable wavelength detector offers low noise and a maximum sensitivity of 0.001 AUFS says Hitachi: both UV and UV/visible versions are available. A \$8,995 (US) Diode Array Detector can be used in conjunction with a PC-based software package for contour plotting, chromatogram overlays and 3-D data display. Alternatively, the system can be used with an integrator which offers complete systems control from one keyboard for single or dual channel operations. The integrator can store up to 600 chromatograms and has data acquisition and data processing capabilities.

Milton Roy LDC Analytical says its 4000 series low-pressure **gradient HPLC systems** offer both high performance and built-in flexibility (*Reader Service No. 115*). The \$14,486 (US) model 4300 system includes a single pump that provides either binary or ternary gradients, a spectroMonitor UV/visible detector, Rheodyne injection valve and C18 column. The \$16,854 (US) model 4400 system includes the above components but features a programmable multi-wavelength detector instead of the spectroMonitor, which the company says has a sensitivity of 0.0005 AUFS. The addition of a PC and LCAdvantage software provides the capability for complete systems control and the capacity for data storage.

### Autosamplers

High sample handling capacity and programmable pre-column derivatization are features of the Gynkotek HPLC **autosampler** from Roth Scientific Company Ltd (*Reader Service No. 116*). The Gina 160 can handle up to 160 samples; the capacity can be increased further by replacing the vials in one of the two sample trays during the run. Injection volumes can be selected from one of two ranges: 1–250 µl or 0.1–25 µl, but vials must contain a minimum volume of 5 µl. Operating



The programmable Gina 160 high-capacity autosampler from Roth Scientific.

parameters for start position, running time, repeat injections and column temperature can be programmed for each sample and are entered via a keyboard and displayed on an LCD. The sample needle is flushed with eluent after each injection to minimize carryover. The autosampler, costing less than £7,000 (UK), can be programmed for pre-column derivatization, where up to two reagents can be added, mixed, and injected into the column after a pre-selected reaction time has elapsed.

Designed for **automated sample transfer**, the new autosampler from Severn Analytical Ltd is compatible with any HPLC system or integrator (*Reader Service No. 117*). The £3,500 (UK) Model 728 uses positive sampling displacement to provide viscosity-independent sample transfer, eliminating the need for accessory gases, says the company. The autosampler can hold up to 64 sample vials and can make up to three injections per vial, rinsing the system after each injection. Sample vials are loaded into the control module before subsequent transfer to a fixed-loop injection valve and injection into the HPLC column. A selection of loop sizes are available, including a variable loop version that allows the user to program a different sample injection volume for each sample. The Model 728 can be used to control integrators and other external components, or can itself be controlled by a PC. □

*These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.*